

REFERENCE ONLY

SHL ITEM BARCODE



19 1727722 3

UNIVERSITY OF LONDON THESIS

Degree

MD

Year

2006

Name of Author

TULLY

Joanna Mary

COPYRIGHT

This is a thesis accepted for a Higher Degree of the University of London. It is an unpublished typescript and the copyright is held by the author. All persons consulting the thesis must read and abide by the Copyright Declaration below.

COPYRIGHT DECLARATION

I recognise that the copyright of the above-described thesis rests with the author and that no quotation from it or information derived from it may be published without the prior written consent of the author.

LOAN

Theses may not be lent to individuals, but the University Library may lend a copy to approved libraries within the United Kingdom, for consultation solely on the premises of those libraries. Application should be made to: The Theses Section, University of London Library, Senate House, Malet Street, London WC1E 7HU.

REPRODUCTION

University of London theses may not be reproduced without explicit written permission from the University of London Library. Enquiries should be addressed to the Theses Section of the Library. Regulations concerning reproduction vary according to the date of acceptance of the thesis and are listed below as guidelines.

- A. Before 1962. Permission granted only upon the prior written consent of the author. (The University Library will provide addresses where possible).
- B. 1962 - 1974. In many cases the author has agreed to permit copying upon completion of a Copyright Declaration.
- C. 1975 - 1988. Most theses may be copied upon completion of a Copyright Declaration.
- D. 1989 onwards. Most theses may be copied.

This thesis comes within category D.

☐

This copy has been deposited in the Library of _____

☐

This copy has been deposited in the University of London Library, Senate House, Malet Street, London WC1E 7HU.

**Risk and protective factors for the development of
meningococcal disease in adolescence:
A biopsychosocial investigation**

Thesis presented for the Doctorate of Medicine

by

Dr Joanna Tully

Institute of Child Health, London

UMI Number: U593543

All rights reserved

INFORMATION TO ALL USERS

The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript and there are missing pages, these will be noted. Also, if material had to be removed, a note will indicate the deletion.



UMI U593543

Published by ProQuest LLC 2013. Copyright in the Dissertation held by the Author.
Microform Edition © ProQuest LLC.

All rights reserved. This work is protected against
unauthorized copying under Title 17, United States Code.



ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Acknowledgments

As with most theses written with the purpose of obtaining a higher degree, this has taken me longer to complete than I first anticipated. For this I must primarily thank my two small children Maya and Manu, whose sleeplessness, fearsome demands, teething episodes and overwhelming loveliness have distracted me from my task these past five years. Offering a more constructive approach to my labours is my husband Sarath who must take the ultimate credit for persuading me that this thesis could be written and completed and thereafter bullying me sufficiently to keep me at my desk. He sacrificed many hours to read chapters of this thesis and provide me with helpful, useful and enlightening suggestions. I am indebted. To my third child, Rudy, I extend my love and grateful appreciation for remaining in-utero long enough for me to undertake the viva examination and subsequent corrections.

A thesis cannot be submitted without guidance from above, and this, along with endless enthusiasm and belief that I would deliver, was provided by Robert Booy my supervisor and mentor. My appreciation of his patience and dedication to research is immense. Russell Viner also provided many hours of useful advice and direction especially concerning strategies for surviving 288 interviews with adolescents. My most heartfelt appreciation is extended towards Pietro Coen, our statistician, without whom I truly believe this thesis would not have reached fruition. I would also like to thank Professor Catherine Peckham for suggesting many additions and changes to the thesis that have made it both more readable and professional.

This piece of work required commitment from many individuals; clinicians, public health professionals and the many young people who agreed to participate. Financial support was generously given by the Meningitis Research Foundation. I would like to extend my thanks to all these individuals without whom this would not have been possible.

And last, but by no means least, I dedicate this thesis to my Mum and Dad who have given up so much to allow me to have achieved so much.

Abstract

Background

The incidence of meningococcal disease (MD) in the United Kingdom increased dramatically from 2.4 per 100,000 in the early 1990's to 4.4 per 100,000 by 1996. The age distribution also shifted towards older teenagers in whom the risk profile for disease is poorly understood and the mortality rate higher. Adolescence is a time of biological, social and psychological change and these changes may contribute towards the risk. This study is a prospective population-based case-control study to identify risk and protective factors for MD in adolescents between the ages of 15 and 19 years.

Methods

Adolescent subjects with MD were recruited at hospital admission in six regions of England from January 1999 to June 2000. One age- and sex-matched control from the same geographical location was recruited per case. Blood samples plus pernasal and throat swabs were taken from cases at hospitalisation and from cases and controls at interview. Data on potential risk and protective factors were gathered at interview.

Results

144 case control pairs were recruited (51% male; median age 17.6) of which 114 cases (79%) were confirmed microbiologically. Significant independent risk factors for MD were history of preceding illness (matched OR 2.9 95% CI: 1.4-5.9), intimate kissing with multiple partners (OR 3.7, 95% CI: 1.7-8.1), being a university student (OR 3.4, 95% CI: 1.2-10) and preterm birth (OR 3.7, 95% CI: 1.0-13.5). Religious observance (OR 0.09, 95% CI: 0.02-0.6) and meningococcal vaccination (OR 0.12, 95% CI: 0.04-0.4) were protective.

Conclusions

This is the first large study examining risk factors for MD within an adolescent population. Activities and events increasing risk for MD in adolescence are different from childhood. Students are at higher risk for disease than their counterparts. While altering personal behaviours may moderate risk of MD, it is unlikely that behaviour-based health promotion can significantly reduce disease burden. The development of effective meningococcal vaccines remains a key public health priority.

Table of contents

1	The pathophysiology, clinical management and outcome of meningococcal disease	15
1.1	<i>Introduction</i>	15
1.2	<i>Pathophysiology</i>	15
1.3	<i>Diagnosis and clinical presentation</i>	16
1.4	<i>Management</i>	17
1.5	<i>Complications and outcome</i>	18
2	The epidemiology of meningococcal disease	19
2.1	<i>Introduction</i>	19
2.2	<i>Notification procedures for meningococcal disease</i>	19
2.3	<i>Epidemiology of carriage</i>	20
2.3.1	<i>Neisseria meningitidis</i>	20
2.3.2	<i>Neisseria lactamica</i>	21
2.4	<i>Epidemiology of invasive disease in the 1980's and 1990's</i>	21
2.4.1	Incidence and age distribution	21
2.4.2	Serogroup and sex distribution	23
2.4.3	Seasonal patterns	24
2.5	<i>Vaccination and changing epidemiology in the 2000's</i>	24
2.6	<i>Summary</i>	25
2.7	<i>Aims of this study</i>	26
3	Biological factors and predisposition to meningococcal disease	27
3.1	<i>Introduction</i>	27
3.2	<i>Respiratory viral infection and meningococcal disease</i>	27
3.2.1	Review of the evidence	28
3.2.1.1	Influenza	28
3.2.1.2	Mycoplasma	29
3.2.1.3	Other respiratory viruses	29
3.2.1.4	"Symptom-based" preceding illness	30
3.2.2	Pathophysiology and proposed mechanisms of action	31
3.2.2.1	Viral infection and transmission	31
3.2.2.2	The effect on nasopharyngeal mucosa	32
3.2.2.3	Viral infection and immunosuppression	33

3.2.3	Relevance to adolescents	37
3.3	<i>Mannose-binding lectin and meningococcal disease</i>	38
3.3.1	Pathophysiology and proposed mechanisms of action	38
3.3.1.1	Structure and function	38
3.3.1.2	Genetics	39
3.3.2	Existing evidence for the role of MBL deficiency in meningococcal disease	39
3.3.3	Relevance to adolescents	40
3.4	<i>Summary</i>	41
3.5	<i>Aims of this study</i>	42
4	Social and behavioural factors and predisposition to meningococcal disease	44
4.1	<i>Introduction</i>	44
4.2	<i>Cigarette smoking and meningococcal disease</i>	45
4.2.1	Review of the evidence	45
4.2.1.1	Smoking and carriage of <i>Neisseria meningitidis</i>	45
4.2.1.2	Smoking and invasive disease	46
4.2.2	Pathophysiology and proposed mechanisms of action	48
4.2.3	Relevance to adolescence	49
4.3	<i>Alcohol, recreational drugs and meningococcal disease</i>	50
4.3.1	Alcohol and meningococcal disease	50
4.3.1.1	Review of the evidence	50
4.3.1.2	Pathophysiology and possible mechanisms of action	51
4.3.1.3	Relevance to adolescence	53
4.3.2	Illicit drug use and meningococcal disease	53
4.3.2.1	Review of the evidence	53
4.3.2.2	Pathophysiology and possible mechanisms of action	53
4.3.2.3	Relevance to adolescents	54
4.4	<i>Overcrowding, intimate contact and meningococcal disease</i>	54
4.4.1	Review of the evidence	54
4.4.1.1	Household overcrowding	54
4.4.1.2	Intimacy, close personal contact and social crowding	56
4.5	<i>Exercise and meningococcal disease.</i>	58
4.5.1	Review of the evidence	58
4.5.1.1	The effect of physical activity on the immune response – possible pathophysiological mechanisms	60
4.5.1.2	Relevance to adolescents	60
4.6	<i>Other social factors and risk of meningococcal disease</i>	61

4.7	<i>Summary</i>	63
4.8	<i>Aims of this study</i>	66
5	Psychological stress, social support networks and susceptibility to infectious disease	67
5.1	<i>Psychological stress & infectious disease</i>	67
5.1.1	Historical perspective	67
5.1.2	Psychological Stress; Definition and concepts	68
5.1.3	Psychological Stress in adolescence	69
5.1.4	Psychological stress and infectious disease; The evidence for a relationship	70
5.1.4.1	Viral Upper Respiratory Tract infection	71
5.1.4.2	Influenza infection	73
5.1.4.3	Latent Virus infections	73
5.1.5	Psychological Stress and meningococcal disease	75
5.1.6	Psychological Stress and infectious disease; A proposed mechanism	76
5.1.6.1	The biological pathway	77
5.1.6.2	The behavioural pathway	78
5.2	<i>Modifiers of the stress-disease relationship; Social support networks</i>	79
5.2.1	The role of social support networks in buffering the effects of stress on the immune system	79
5.2.2	Social support networks in adolescence	83
5.2.3	Other moderating variables	83
5.3	<i>Summary</i>	84
5.4	<i>Aims of this study</i>	84
6	Methods	86
6.1	<i>Study design</i>	86
6.2	<i>Study protocol</i>	88
6.2.1	Multi-centre design	88
6.2.2	Issues of collaboration	88
6.2.2.1	Consultants in Communicable Disease Control	88
6.2.2.2	The Manchester Reference Laboratory	89
6.2.2.3	Hospital clinicians	89
6.2.2.4	Meningitis Research Foundation	90
6.2.3	Development of the questionnaire	90
6.2.3.1	Part 1 - Questionnaire for young people	91
6.2.3.2	Part 2 - Questionnaire for Head of Household	99
6.2.3.3	The questionnaire and recall bias	101
6.2.3.4	Validation issues	101

6.3	<i>Ethics approval</i>	102
6.4	<i>Subject recruitment</i>	102
6.4.1	Case Recruitment	102
6.4.1.1	Ethical considerations in case recruitment	103
6.4.1.2	Exclusion criteria	104
6.4.2	Control recruitment	104
6.5	<i>Interviewing subjects</i>	107
6.6	<i>Laboratory specimens</i>	108
6.6.1	Collection from subjects	108
6.6.1.1	Case subjects	108
6.6.1.2	Control subjects	109
6.6.2	Specimen handling	110
6.6.3	Respiratory viral studies	111
6.6.3.1	Influenza Virus	111
6.6.3.2	Epstein Barr Virus	112
6.6.3.3	Respiratory Syncytial Virus	112
6.6.3.4	Chlamydia and Mycoplasma	113
6.6.4	Mannose Binding Lectin	113
6.6.5	Meningococcal serology	113
6.6.6	Temporal difference in sample collection	113
6.7	<i>Data handling and analysis</i>	115
6.7.1	Formic Automatic Data Capture	115
6.7.2	Data checking and database design	115
6.7.3	Statistical analysis of data	116
6.8	<i>Difficulties encountered with the study</i>	116
6.8.1	Subject recruitment	116
6.8.1.1	CCDC non-collaboration	117
6.8.1.2	GP non-collaboration	117
6.8.1.3	Late referrals	117
6.8.2	Subject numbers	118
6.8.2.1	The effect of introduction of the vaccine	118
6.9	<i>Bias and case control studies</i>	118
6.9.1	Selection bias	119
6.9.1.1	Case selection	119
6.9.1.2	Control selection	121
6.9.2	Observation or information bias	121
6.9.2.1	Interviewer bias	121
6.9.2.2	Recall bias	122

6.9.2.3	Social desirability bias	123
6.9.2.4	Other possible bias	123
7	Results	124
7.1	<i>Recruitment</i>	124
7.1.1	Case recruitment	124
7.1.1.1	Reasons for non-recruitment	124
7.1.1.2	Source of recruitment	125
7.1.2	Control recruitment	125
7.2	<i>Demographic characteristics of subjects</i>	125
7.3	<i>Univariate analysis results</i>	128
7.3.1	Biological hypotheses	128
7.3.1.1	Preceding illness	128
7.3.1.2	Mannose-binding lectin	129
7.3.1.3	Other biological factors	129
7.3.2	Social hypotheses	132
7.3.2.1	Substance use – cigarettes, alcohol and drugs	132
7.3.2.2	Intimate and social contact and leisure behaviours	134
7.3.2.3	Other social behaviours	136
7.3.3	Psychological hypotheses	138
7.3.3.1	Life stress and social support networks	138
7.4	<i>Multivariate analysis</i>	140
8	Discussion	142
8.1	<i>Summary of findings</i>	142
8.2	<i>Strengths of this study</i>	142
8.3	<i>Comparisons with previous work</i>	143
8.3.1	Biological factors	143
8.3.1.1	Preceding illness	143
8.3.1.2	Mannose-binding lectin	146
8.3.1.3	Other biological factors	147
8.3.2	Social factors	147
8.3.2.1	Substance use	147
8.3.2.2	Social contact & leisure behaviours	149
8.3.2.3	Exercise	150
8.3.2.4	Others	150
8.3.3	Psychological factors	152
8.3.3.1	Stress	152

8.3.3.2	Social support	153
8.4	<i>Limitations of this study</i>	153
8.5	<i>Conclusions</i>	158
9	References	160

Table of tables and figures

Figure 1	Laboratory confirmed meningococcal infections by age group	22
Figure 2	Mortality from meningococcal infection 1993 – 2002	24
Figure 3	Case recruitment patterns	120
Figure 4	Recruitment by region	125
Table 1	Sample size calculations	87
Table 2	Demographic data	127
Table 3	Univariate analysis – biological factors excluding MBL	130
Table 4	Univariate analysis – MBL	131
Table 5	Univariate analysis – substance use behaviours	133
Table 6	Univariate analysis – social contact, leisure and intimacy behaviours	135
Table 7	Univariate analysis – other social variables	137
Table 8	Univariate analysis – life stress and social support	139
Table 9	Multivariate analysis of risk factors for meningococcal disease	141

Table of appendices

Appendix 1	Introductory letter to CCDCs'	181
Appendix 2	Introductory letter to consultants	183
Appendix 3	Introductory letter to medical directors	185
Appendix 4	Introductory letter to intensive care units	187
Appendix 5	Introductory letter to microbiologists	189
Appendix 6	Introductory letter to GP	190
Appendix 7	Case notification form	192
Appendix 8	Recruitment checklist	193
Appendix 9	General information sheet	195
Appendix 10	Case information sheet	197
Appendix 11	Control information sheet	202
Appendix 12	Relative information sheet	207
Appendix 13	Case consent form	209
Appendix 14	Control consent form	211
Appendix 15	Next-of-kin consent form	212
Appendix 16	Instruction sheet	214
Appendix 17	Checklist for GP pack	216
Appendix 18	GP letter to control	217
Appendix 19	Control identification form	219
Appendix 20	Control response form	221
Appendix 21	Thanks to case consultant	222
Appendix 22	Thanks to GP	225
Appendix 23	Thanks to subject	226

Appendix 24	Study questionnaire	227
Appendix 25	Risk and protective factors for meningococcal disease in adolescents: a matched cohort study	
	BMJ vol 332 Feb 25 2006	269
Appendix 26	Effectiveness of meningococcal C conjugate vaccine in teenagers in England	
	The Lancet vol 361 Feb 22 2004	288
Appendix 27	The new system of review by multicentre research ethics committees: prospective study	
	BMJ vol 320 April 29 2000	290
Appendix 28	Is it exposure to cigarette smoke or to smokers which increases the risk of meningococcal disease in teenagers?	
	Int J Epi vol 35 April 2006	294

Introduction

Acute infection with *Neisseria meningitidis* claims the lives of around 10% of those affected. Most affected individuals are previously healthy children and adolescents. Although typically a disease of childhood, during the decade that this study was conceived there was a marked increase in the number of young adolescents developing and dying from meningococcal disease.

Adolescence is a period of human development characterised by marked biological, social and psychological change. Bearing in mind the risk factors for meningococcal disease already elucidated in previous work and outlined in forthcoming chapters of this thesis, and considering the unique adolescent lifestyle, a set of hypotheses were generated in an attempt to determine factors associated with adolescent susceptibility to meningococcal infection.

These hypotheses form the basis for the investigation presented here. The following chapters will explore the background to the development of the hypotheses, drawing extensively on previous literature surrounding the subject. The methodology employed to answer the questions posed will be fully explained and the results obtained presented. Finally a full discussion of the interpretation and implications of these results to the fields of infectious disease medicine and public health will be offered.

Outlined below are the 6 main hypotheses that this study set out to investigate.

Biological hypotheses

- preceding illness with upper respiratory tract infections, in particular influenza A and B virus and Epstein Barr Virus, but also Respiratory Syncytial Virus, mycoplasma and chlamydia, predispose teenagers to a greater risk of MD.
- deficiency of Mannose Binding Lectin predisposes adolescents to MD.

Social hypotheses

- the risk of MD in late adolescence is related to developmental social factors such as mouth kissing, substance use and changing social interaction patterns.
- certain leisure activities, including involvement in elite athletic pursuits, are associated with an increased risk of MD.

Psychological hypotheses

- adolescent life stress predisposes to MD
- inadequate social support is associated with an increased risk of MD.

1 The pathophysiology, clinical management and outcome of meningococcal disease

1.1 Introduction

Despite advances in vaccine technology and paediatric intensive care management, meningococcal disease still remains a worldwide problem and a leading cause of mortality in children in the developing world. The pathophysiological processes leading to the clinical spectrum of disease observed have been elucidated relatively recently. The centralisation of care of critically ill individuals along with greater awareness of the disease and the necessity for early recognition and treatment have led to decreasing mortality in recent years [¹]. The mainstay of management is early and aggressive fluid therapy and recognition of raised intracranial pressure, and many of these patients require intensive care treatment. Although medium-term complications of the disease are common, longer-term outcomes are generally favourable in those who survive.

1.2 Pathophysiology

Neisseria meningitidis is a gram-negative coccus belonging to the family Neisseriaceae. The organism classically occurs in pairs and may be either encapsulated or non-encapsulated. The presence of a polysaccharide capsule protects the organism from phagocytosis. Just inside the capsule is the outer cell membrane containing proteins involved in adherence and invasion. On the surface are pili that are also important in adherence, colonisation of the host and invasion. Antigenic variation in these components allow the meningococci to resist host immune responses [²].

Meningococci can be divided into serogroups, serotypes, subtypes, immunotypes and multilocus enzyme electrophoresis types depending on constitutional differences in the organism [²]. Thirteen different serogroups have been identified; A, B and C being the commonest causes of invasive disease worldwide [²].

Neisseria meningitidis colonises the human nasopharynx, genital tract and eye by adherence to the mucosal surface. Colonisation of the nasopharynx is important as a

source of transmission to other persons and as a site of invasion leading to systemic disease. The bacterium has a number of mechanisms enabling it to avoid host immunological responses in the nasopharynx, allowing it to pass through the nasopharyngeal membrane and into the bloodstream.

Once in the bloodstream the host immune response is activated. It is the activation of host immunity that appears to lead to much of the damage to host tissue and the clinical spectre observed [3]. Amongst other chemicals generated, meningococci cause the release of large quantities of endotoxin into the bloodstream [3]. Endotoxin binds to a number of host inflammatory cells, triggering an intense inflammatory process.

During this inflammatory cascade there is a profound change in the finely regulated functions of the microvasculature and it is these changes that lead to the severe physiological consequences observed [3]. The vascular endothelium regulates vascular permeability, and disruption to this process by invading meningococci leads to increased vascular permeability, vasoconstriction and vasodilation, intravascular coagulation and myocardial dysfunction [3]. This combination of pathophysiological processes can result in the presentation of a severely ill and rapidly deteriorating individual.

1.3 Diagnosis and clinical presentation

Meningococcal infection still remains an important cause of mortality and morbidity in children worldwide. Mortality has fallen over recent years [1;4;5], mostly due to centralisation of care in paediatric intensive care units, increased early recognition of severe disease and development of treatment protocols [4;6].

Meningococcal infection typically manifests as septicaemia (10% of cases), meningitis (50% of cases) or a mixed picture (40% of cases), although other rarer presentations are seen [4]. Septicaemic children present with fever, a typically non-blanching rash, vomiting, myalgia, abdominal pain, tachycardia, cool peripheries and hypotension. Children with meningitis may have headache, fever, vomiting, photophobia, lethargy and neck stiffness.

The initial diagnosis of meningococcal disease is normally a clinical one as immediate institution of treatment is required, usually prior to the availability of laboratory results. Treatment should not be delayed in order to obtain diagnostic samples. Blood for culture is not particularly sensitive, especially if antibiotics have been administered prior to arriving at hospital. Other diagnostic options from blood are polymerase chain reaction (PCR) for meningococcal DNA, latex agglutination test and acute and convalescent serology. In addition culture from throat swabs or skin scrapings from purpuric lesions may yield a positive result [4]. Diagnosis of meningitis using lumbar puncture is unlikely to alter initial management and should only be done if the recognised contraindications are absent. It may be more safely performed later in the course of disease and might be useful in confirming the diagnosis and informing medium-term treatment and follow-up.

1.4 Management

The management of children suspected of having meningococcal disease requires rapid diagnosis and the immediate administration of antibiotic therapy [7]. Senior doctors should be involved in their care from an early stage as progression of the disease can be rapid. Assessment of airway, breathing and circulation should be made and resuscitation instigated as appropriate. Circulatory shock and raised intracranial pressure (ICP) form the main immediate threat to life and the risk of developing these complications should be constantly reassessed.

Recognition of shock and aggressive early fluid therapy are vital and can be life-saving [4,6]. Patients with tachycardia, tachypnoea, metabolic acidosis, reduced urine output, hypoxia and cool peripheries need fluid bolus therapy. The type of fluid used has been recently subject to much controversy [8] but can be either normal saline or human albumin [4].

Patients with meningitis may present with features of raised intracranial pressure including deteriorating conscious level, normal or high blood pressure, pupillary abnormalities, focal neurology or seizures [4]. These patients need immediate intensive care management to optimise cerebral blood flow.

A proportion of children presenting with this disease will require management on intensive care units where shock, multi-organ failure and raised ICP remain the main

management challenges. Other patients can be safely managed on a general paediatric ward with close monitoring. Uncomplicated disease can be treated with seven days of intravenous antibiotics. If this is with penicillin alone (as opposed to a third generation cephalosporin), oral rifampicin is also required to eliminate nasopharyngeal carriage [4;6]. Close contacts should also receive prophylactic antibiotic therapy and immunisation if indicated.

Novel therapies are constantly being sought in attempts to overcome the continuing high mortality of children and adolescents with severe meningococcal disease. Many of these therapies seek to modulate the inflammatory process, but as yet are not part of the mainstay of management [4;9].

1.5 Complications and outcome

Reports on mortality rates for children requiring intensive care vary between 2% [1] and 35% [9]. Patients on intensive care, who by definition are severely ill, are vulnerable to medium-term complications related to the disease process and to iatrogenic complications arising from prolonged intensive care stays. These include neurological complications such as seizures, hemiplegia and deafness, renal complications, in particular renal failure, requiring prolonged haemofiltration or peritoneal dialysis, coagulation disorders and complications arising from these, as well as the possibility of fasciotomies or amputations resulting from necrotic areas of skin or limbs [4]. Prolonged ventilation may necessitate tracheostomy. Post-traumatic stress disorder had been recognised in both the patient and parents [10].

Despite this, in the longer term most patients survive meningococcal disease intact, even those who were critically ill. However, about five percent of survivors have permanent neurological sequel, most commonly sensorineural deafness. Limb or finger ischaemia leading to amputation or skin grafting occurs in a further two to five percent. Rarely renal or myocardial dysfunction persists into the longer term [4].

2 The epidemiology of meningococcal disease

2.1 Introduction

Meningococcal disease remains a significant public health problem in both developed and developing countries. It is an infection that occurs in previously healthy people of all ages and carries a high mortality. During the 1990's changes in the epidemiology of meningococcal disease in Europe and the United Kingdom were reported [^{11;12}]. An increase in the overall incidence of disease along with a shifting age distribution towards adolescents was noted. This provided the impetus for the investigation reported in this thesis.

2.2 Notification procedures for meningococcal disease

Throughout the 20th century, *Neisseria meningitidis* has produced intermittent epidemics of disease throughout the United Kingdom. As early as 1912 acute bacterial meningitis became a notifiable disease. For over 30 years clinicians have been notifying cases of meningococcal meningitis to their local public health authorities and, since 1989, also cases of meningococcal septicaemia. In 1987 a surveillance system was introduced in Europe, including England, where contributors provided information on all reported cases of meningococcal disease in their country [¹³]. This surveillance system was set up in response to reports of changing epidemiology of meningococcal disease in Europe in order to monitor and anticipate further changes.

Surveillance of meningococcal infection in England and Wales relies on three data sources. Firstly, cases diagnosed clinically must, by statute, be notified to the local Consultant in Communicable Disease Control (CCDC) who notifies the Health Protection Agency (HPA), formerly the Public Health Laboratory Service's Communicable Disease Surveillance Centre (CDSC). Secondly clinical samples and isolates are forwarded to the Meningococcal Reference Unit (MRU) in Manchester for diagnosis confirmation and further typing. Lastly death registrations listing meningococcal disease as the most probable cause of death are sent to the Office of

National Statistics, where they are coded according to the *International Classification of Diseases* [14].

The data derived from this surveillance provide information that can be used to monitor trends in the epidemiology of meningococcal disease in the United Kingdom.

2.3 Epidemiology of carriage

2.3.1 *Neisseria meningitidis*

As previously mentioned *Neisseria meningitidis* is a gram-negative diplococcus that colonises the human nasopharynx. Once established it exhibits either silent carriage in the upper respiratory tract or an overt invasive disease process.

Nasopharyngeal carriage of *Neisseria meningitidis* is a prerequisite for invasive disease. However, certain features of the epidemiological pattern of meningococcal carriage indicate that it is not the sole risk factor for disease. Firstly, carriage rates vary with age in a different manner than invasive disease rates vary with age (see below). Secondly, invasive disease incidence varies by season whilst carriage incidence does not [15]. Finally, in a given population two incongruous patterns of infection can be identified – high carriage rates associated with relatively low case rates (adolescents) and low carriage rates with high case rates (infants) [16]. So whilst carriage rates are important, they are not the only factor determining disease in a population.

Overall carriage rates within the population are in the region of 10% [16]. However, carriage rates vary significantly with age. Principally, a low carriage rate is found in children under 5 years (despite this being the peak age for invasive disease), a peak in carriage rates identified in young adults (who have lower invasive disease rates) after which carriage rates steadily decline with increasing age (as do invasive disease rates with the exception being the elderly who have slightly higher rates)[16]. In Cartwright's survey including over 6,000 swabs a carriage rate of nearly 25% was identified in the 15 to 19 year age group whereas only 2% of the under 5's were carriers. Males outnumbered females by 3:2. The duration of carriage once

colonisation has occurred is probably highly variable, although median durations between 9.6 and 14.5 months seem to be the most frequently reported [15].

2.3.2 *Neisseria lactamica*

Neisseria lactamica is a related bacterium that shares the same colonisation site with the meningococcus but is non-pathogenic. It is thought to be important in the development of immunity to meningococcal carriage and disease by inducing antibodies that cross-react with and protect against meningococci [17]. The highest carriage rates of *lactamica* occur in young children, peaking in the latter half of the second year of life when carriage prevalence of *Neisseria meningitidis* is lowest [16;17]. Whilst a male preponderance is observed with meningococcal carriage, female carriers of *Neisseria lactamica* outnumber males in older children and adults. The duration of carriage with *Neisseria lactamica* is shorter than that with *Neisseria meningitidis* but the protection against invasive meningococcal infection afforded by *Neisseria lactamica* acquisition lasts an average of 4.7 years [15].

2.4 Epidemiology of invasive disease in the 1980's and 1990's

The epidemiology of meningococcal disease in the developed world represents a basically stable endemic situation with occasional outbreaks occurring against this background. Since the middle of the 1980's and into the late 1990's the incidence of meningococcal disease in England and Wales increased. In addition changes were noted in both the age-specific distribution and the serogroup of meningococcus causing infection [18].

2.4.1 Incidence and age distribution

The overall incidence of meningococcal disease in England and Wales in the early 1990's was estimated to be 2.4 per 100,000 population [13]. Between 1994 and 1996 notifications of meningococcal disease rose from 1129 cases to 2330 cases [19]. By 1996 the reported incidence was 4.4 per 100,000 population [14].

The age distribution of cases has followed a fairly consistent pattern throughout the past two decades, with one important exception. The general pattern reported by Jones in 1993 is that neonates have a rate of 5.9 per 100,000 after which a steady increase in incidence is seen with each month of life, peaking at the age of 6 months (52 per 100,000). Rates then decline sharply to reach a nadir at age 10 years (1.2 per 100,000). A second smaller peak is seen at age 17-18 years (4.8 per 100,000) after which adult average rates of 0.4 per 100,000 are soon reached (see figure 1) [18]. Generally about half of all cases of invasive meningococcal disease occur in children under the age of five years [13].

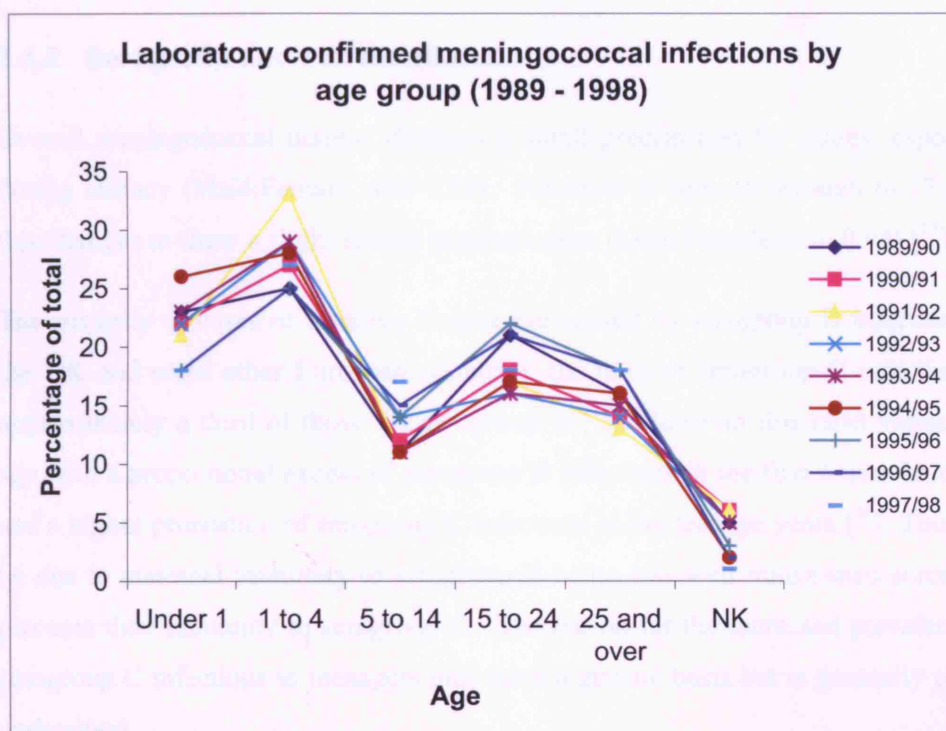


Figure 1 Graph illustrating the smaller secondary peak in incidence occurring in the late adolescent age group.

Figures from Ramsay et al [14]

The important exception to this consistent pattern of age distribution in incidence throughout the 1980's and 1990's was noted in the second half of the 1990's. The general increase in incidence reported at this time was associated with a shift in age distribution away from the under 5's towards the 15 to 19 year age group [13;14].

Teenagers and young adults are also over-represented during epidemics when the age-distribution pattern changes [13].

The rates of meningococcal disease for infants in the early weeks of life reflect the general vulnerability of the neonate to infection balanced by the presence of maternal antibodies. The steady increase in incidence that follows almost certainly reflects the gradual waning of the protection afforded by maternally-derived immunity during the first 6 months. Natural immunity is rapidly acquired following the peak susceptibility around 6 months of age. The development of the second albeit smaller peak in adolescence requires further explanation [18].

2.4.2 Serogroup and sex distribution

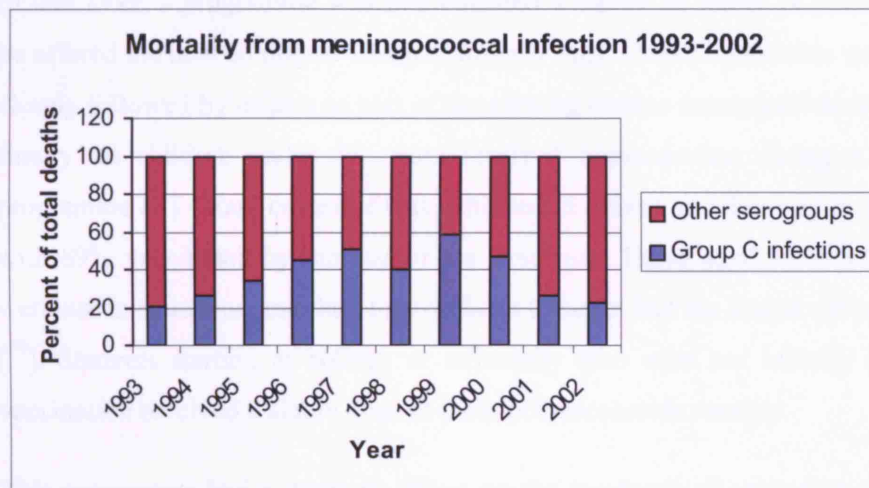
Overall meningococcal disease displays a small predilection for males, especially during infancy (Male:Female ratio 1.39). However at ages 15 through to 17 years this changes to show a slight female predominance (Male:Female ratio 0.94) [18].

The majority of cases of invasive disease are caused by serogroup B infections. In the UK and some other European countries, the rates of serogroup C infection are approximately a third of those for serogroup B [18]. However this ratio varies with age with a proportional excess of serogroup B infections in the first 6 months of life and a higher proportion of serogroup C infections in the teenage years [18]. This may be due to maternal immunity to serogroup B being less well transmitted across the placenta than immunity to serogroup C. The reason for the increased prevalence of serogroup C infections in teenagers may have a genetic basis but is generally poorly understood.

Alongside the general increase in incidence of MD in 1995, a significant increase in the proportion of infections due to serogroup C was noted [13;14], a substantial proportion of these in teenagers. Outbreaks of an epidemic strain C:2a:P1.2 were experienced in England as well as other countries in Europe, eg. the Czech Republic and Greece [13]. When such a new strain emerges, to which the general population lacks immunity, serogroup C disease carries with it a higher case-fatality rate than serogroup B (see figure 2) [14;20]. In the years 1993 to 1998 in England and Wales, over 400 people died from serogroup C meningococcal disease alone [21]. A further 1,767 required intensive care admission, most of these being children and

adolescents [21]. Although studies vary, up to 45% of those surviving serogroup C disease may develop sequelae [21]. This represents a substantial public health problem.

Figure 2 The rising contribution to mortality made by serogroup C infections in the second half of the 1990's



Data from El-Bashir et al [22].

2.4.3 Seasonal patterns

Meningococcal disease follows a known seasonal pattern with cases being commoner in the winter than summer, the highest number of infections occurring in January [18]. This is largely independent of age although Jones' data suggest that in November and December there were proportionately more infections in the adolescent age group [18].

2.5 Vaccination and changing epidemiology in the 2000's

Due to concern at the increasing incidence of invasive meningococcal disease in England and Wales during the mid to late 1990's, coupled with the increase in serogroup C disease and its higher case-fatality rate, a decision was made by the UK government to introduce widespread vaccination against serogroup C meningococcal disease.

Prior to the development of the conjugate C vaccine, the only available vaccine was the plain polysaccharide vaccine. This vaccine does not protect infants and toddlers and only confers short-lived immunity [23]. Once the polysaccharide is conjugated to a protein carrier the vaccine becomes more effective in children and the immunity conferred more durable.

In late 1999, a programme was implemented whereby all under 18 year-olds would be offered the new conjugate vaccine. Initially only 15 to 17 year-olds were targeted, closely followed by infants as part of the existing routine immunisation schedule, and finally all children under 18 years received immunisation during a “catch-up” programme [24]. Good coverage was achieved in infants and those up to 14 years old with 89% vaccinated by the end of the first year. Those aged 15 to 17 years who were not in education and therefore difficult to target had the lowest coverage at 43% [25]. Students starting at college or university who were not initially targeted for vaccination received a single dose of plain polysaccharide vaccine.

This programme had a dramatic effect on the incidence of serogroup C disease in 2000 through to 2001. During this winter period the incidence of serogroup C disease fell by 80% and the number of deaths in children under 19 years fell from 78 in 1998/1999 to just 8 in 2000/2001 [25].

2.6 Summary

Meningococcal disease remains a public health problem in developed as well as developing countries. Carriage of the pathogenic bacteria is a prerequisite for invasive disease, but is not the sole risk factor. Carriage rates vary with age, the highest rates being seen in those aged 15 to 19 years. Acquisition of the related but non-pathogenic *Neisseria lactamica* can confer protection as can carriage of *meningitidis* itself.

Meningococcal disease is mainly a disease of young children, with an incidence peak around the age of 6 months. A second smaller peak is observed during adolescence. Generally the disease exhibits a slight male preponderance and is more common during winter months. Disease caused by serogroup B is the most common in the United Kingdom, although serogroup C disease accounts for about a third of cases.

During the mid-1990's a dramatic increase in the incidence of invasive disease was noted in England and Wales. In particular an increase in the proportion with serogroup C disease occurred. This accompanied a shift in age distribution away from the under fives towards the teenage group. As serogroup C disease carries a higher mortality than serogroup B disease, this was a significant cause for concern and provided the impetus for the introduction of the conjugate serogroup C vaccine in 1999. This dramatically decreased both the incidence and the mortality rate from invasive serogroup C meningococcal disease in young people.

2.7 Aims of this study

Although carriage rates of meningococci reach a peak during teenage years, this cannot fully explain why a small but definite peak in incidence of invasive disease is seen in a population of young immunocompetent individuals. Meningococcal disease has the special ability of an infectious agent in today's developed world to confer high mortality on previously healthy individuals. An explanation as to why teenagers have a relatively and unexpectedly high incidence of meningococcal disease has not been forthcoming. Investigating the risk factors for the development of this disease within this specific population may help to increase our understanding of this observed phenomenon.

3 Biological factors and predisposition to meningococcal disease

3.1 Introduction

In this chapter the role of two biological factors in the predisposition to meningococcal disease are examined. Firstly that of preceding respiratory viral infection, and secondly that of one component of the innate immune system, mannose-binding lectin. The existing evidence for an association will be explored, the pathophysiology of any observed effect explained and the relevance to disease in adolescence outlined.

3.2 Respiratory viral infection and meningococcal disease

Considerable epidemiological and experimental evidence exists suggesting that primary viral infection is an important predisposing event for both local and systemic bacterial infection. Probably the first recorded observation to this effect was made by a German physician, Clemens von Pirquet in 1908 [26]. He noted that infection with an agent now known to be measles virus could lead to subsequent infection with other organisms, complicating the clinical picture.

Since this time the notion that primary viral infection could predispose to secondary bacterial invasion has been much studied. The evidence for such an association had been most compelling for respiratory viral infections. Such infections are common during early childhood [27] coinciding with the peak incidence of meningococcal disease. Many respiratory viruses share a seasonal pattern with meningococcal disease – being more common during the winter months. Therefore the proposed relationship between respiratory viral infection and the subsequent development of meningococcal disease has merited investigation and careful analysis in order to address this ecological observation.

3.2.1 Review of the evidence

3.2.1.1 Influenza

The observation that influenza epidemics were associated with an increase in the number of cases of meningococcal disease led to initial observational work elucidating a temporal relationship between influenza A infection and meningococcal disease. This was carried out in relatively small numbers of patients during localised outbreaks [28-30]. Lal et al reported simultaneous episodes of influenza and meningococcal infections in military recruits [28], Young and colleagues noted the association in an institution for middle-aged mentally ill women [29] whilst Harrison et al studied a cluster of meningococcal cases on a school bus following an influenza outbreak [30]. Although serological techniques for confirmation of influenza infection were employed in these studies, numbers included were small, proper control groups were not always present and results are therefore difficult to interpret reliably.

However further epidemiological work using larger numbers of subjects still suggested this relationship might exist [31-33]. During a major epidemic of serogroup A meningococcal meningitis in Chad, Moore et al [32] demonstrated a significantly increased chance of respiratory viruses being present in case patients than in matched controls. This relationship was especially strong in those over the age of 15 compared to younger children. The authors concluded that further investigation was necessary in developed countries.

An outbreak of meningococcal disease in the United Kingdom in 1989 provided this opportunity. The resulting publication demonstrated that patients suffering from meningococcal disease were nearly four times as likely to show serological evidence of recent influenza infection than were controls [31]. Interestingly it was also suggested that this effect was greater in older children or adolescents than in a younger age group.

Following this, in 1996 Watson et al [33] examined surveillance data for England and Wales in the light of these preliminary reports. In order to overcome the fact that a correlation would be likely between the two diseases based on season of occurrence alone (as both are winter diseases), five years of data were deseasonalised and

analysed for association. A strong correlation was still observed between the two infections, reducing the likelihood that the noted association was a spurious one due to seasonal coincidence.

3.2.1.2 Mycoplasma

Having demonstrated an epidemiological link between Influenza A and meningococcal disease, the wider concept of the relationship with other respiratory pathogens was explored.

The study by Moore et al conducted in Chad during the 1988 epidemic of meningococcal meningitis, demonstrated a clear relationship between meningococcal disease and isolation of mycoplasma species, despite the fact that the study was not originally designed to detect this relationship. Again this was most pronounced in the older age group of patients approaching adulthood [32]. Kleemola et al [34] reported a four-fold rise in complement fixation titers against *Mycoplasma pneumoniae* in meningitis patients, a significantly more frequent finding than in the control population. Krasinski et al [35] studied patients admitted with bacterial meningitis and found that they were much more likely to have antibody to *M. pneumoniae* than their control group. It is possible that these results may be due to non-specific cross-reaction with tissue antigens released during tissue injury and therefore cautious interpretation of serological results for mycoplasma infection is reasonable [34]. Newer techniques for more accurate diagnosis of infection such as polymerase chain reaction should give a clearer picture of any possible association.

3.2.1.3 Other respiratory viruses

Although influenza virus has been the most widely studied and provides the most compelling evidence for a link, there is scanty evidence for a similar role amongst other viruses. It is now widely accepted that Cytomegalovirus (CMV) and Human Immunodeficiency virus (HIV) predispose the host to a variety of opportunistic infection, but the evidence for a similar action in other more common viruses, especially in relation to meningococcal disease, is lacking.

A small body of evidence suggests that other viruses may predispose the host to secondary infection. Adenovirus, Parainfluenza virus, Varicella Zoster virus and Respiratory Syncytial Virus (RSV) have all been associated with an increased risk of

development of secondary bacterial otitis media and pneumonia [^{35;36}]. RSV in particular is a common winter respiratory virus amongst infants and young children. In the light of the evidence surrounding Influenza A, and the finding that meningococci have an increased affinity for RSV-infected Hep2 cells in vitro [³⁷], the possibility of an association between RSV infection and meningococcal disease was evaluated by Stuart et al in 1996 [³⁸]. The descriptive epidemiological study performed failed to show a predisposition to meningococcal disease in those infected by RSV although to date no further work has been undertaken to refute or confirm these findings.

Epstein Barr Virus (EBV) is the cause of infectious mononucleosis or glandular fever. It is known to be an immunosuppressive virus (see section 3.2.2.3.1). It commonly infects young children in the developing world (usually giving rise to mild or subclinical disease), and adolescents in the developed world where it typically causes full-blown glandular fever [³⁹]. Very few publications explore possible associations between EBV infection and subsequent bacterial infection. EBV titre rises in conjunction with pertussis, hepatitis and meningitis have been documented [⁴⁰], but the implications of this observation are unclear. There are no published data on the association between EBV and meningococcal disease.

3.2.1.4 “Symptom-based” preceding illness

Few previous studies have examined preceding illness in terms of specific viruses as discussed above, but most have focused on a cluster of symptoms indicating general respiratory infection [⁴¹⁻⁴⁴]. The definition of preceding illness using this approach has varied between studies and as might be expected, results vary. Most studies employed the presence of a defined number of symptoms out of a predefined collection of symptoms as being indicative of respiratory viral infection. For example, Bruce et al used two or more out of four common symptoms found during respiratory infection to indicate its presence [⁴¹], while Stanwell-Smith et al used a combination of any three out of five symptoms [⁴³]. Haneberg was less specific using “general symptoms of ill-health” as his indicator [⁴⁴] while Moodley used only the presence of a congested nose to indicate respiratory infection [⁴²]. In addition, the time frames prior to meningococcal disease developing, within which these symptoms had to be present, also vary between studies. Both Stanwell-Smith and

Haneberg used a two-week period, while Moodley and Bruce used a one-month period. Two of the studies use subjects from all age groups [^{43;44}], while Bruce only examined college students [⁴¹] and Moodley only children under 14 years [⁴²].

Not surprisingly given this heterogeneous comparison, varying results have been achieved. Both Bruce and Haneberg found preceding illness to be a significant risk factor for disease while Moodley only found it to reach significance when in conjunction with passive smoke exposure. Stanwell-Smith found that preceding illness was not significantly associated with disease.

Apart from the fact that comparisons between the above studies are difficult, there is one other important consideration when approaching preceding illness in this manner. Meningococcal disease is often preceded by a prodromal illness that can appear very similar to a flu-like upper respiratory infection in terms of symptoms. It could be postulated that using a symptom-based definition for preceding illness might lead to a false association between meningococcal disease and preceding illness if in fact it was the prodromal symptoms that were being used as indicative of a preceding illness. The further in time the preceding illness is from the episode of meningococcal disease, the less likely it is to be the prodromal illness. However none of the above studies specified the median length of time between onset of the preceding illness and the episode of meningococcal disease.

3.2.2 Pathophysiology and proposed mechanisms of action

Following these observations it is interesting to postulate the mechanism by which respiratory viral infection might predispose to meningococcal disease. Three main areas provide potential explanations.

The first is that a concomitant viral infection may increase *transmission* of meningococci, the second explores the effects of viruses on *nasopharyngeal mucosal integrity* and the third looks at the effects of viral infection on the *immune system*.

3.2.2.1 Viral infection and transmission

It may seem intuitive that the symptoms that often accompany viral infection, such as sneezing and coughing, may lead to an increase in transmission of meningococci from person to person. However carriage rates do not increase during the season

when upper respiratory tract infection is prevalent [31]. In addition a prominent feature of RSV infection in young children and infants is cough, and yet Stuart et al failed to show any relationship between RSV and meningococcal disease [38]. It seems unlikely that increased transmission during symptomatic illness is an important factor in explaining the observed relationship.

3.2.2.2 The effect on nasopharyngeal mucosa

The second theory that has been put forward is that preceding viral infection may increase the invasive potential of meningococci through enhanced binding of bacteria to virus-infected epithelial cells and through damaging nasopharyngeal mucosal integrity. The nasopharynx has long been implicated as the natural habitat of the meningococcus and as the site of invasion preceding systemic disease. A prominent feature of many respiratory viral infections is sore throat accompanied by nasal congestion and discharge [45]

Research done by Stephens et al demonstrated the interaction of *Neisseria meningitidis* with a small number of non-ciliated columnar epithelial cells of the human nasopharyngeal mucosa [46]. High concentrations of receptors for meningococcal pili on cells of the nasopharynx were documented. This may explain the tendency of the meningococcus to attach selectively to this site. Subsequent to this, they were able to demonstrate phagocytosis but not destruction of bacteria by this limited population of non-ciliated columnar cells. The authors postulated that meningococci were then transported through to sub-epithelial tissues by the same cells acting as mediators of bacterial transport.

This provides us with a theoretical model of the way in which the meningococcus may penetrate the mucosa to cause overt disease. Stephens et al did not, however, examine the effects of viral infection on the specific mucosal cells believed to be involved in the interaction with meningococcus, although current theory supposes that local tissue damage allows rapid and more efficient invasion.

Stephens et al observed distant damage to uninfected cells of the nasopharyngeal mucosa to which meningococci were not attached suggesting a toxin-mediated effect on other nasopharyngeal cells by the bacterium. These damaged cells were noticed to have significantly decreased ciliary action and it could be argued that such damaged

cells might be more permissive to invasion. If viral infection caused similar damage to ciliary function then it too would allow more ready access to the systemic circulation.

If enhanced binding and lowered resistance to invasion does indeed predispose to invasive meningococcal disease, one would expect to find a higher rate of isolation of viruses from patients with meningococcal disease than from controls.

Moore et al [³²] found a viral or mycoplasma agent in nasopharyngeal washings in 47% of patients with meningococcal disease compared to 11% of controls. However, no influenza viruses were isolated from either group as the study was performed during a period of low influenza activity. Krasinski and colleagues [³⁵] found documented evidence of viral infection in 40% of patients admitted to hospital with bacterial meningitis. However the control group was selected from children admitted for elective surgery and these children tend to be healthier than the general population.

Although no definitive association has been demonstrated between RSV infection and clinical meningococcal disease [³⁸], the work by Raza et al [³⁷] demonstrated enhanced binding of meningococci to RSV-infected human epithelial cells.

3.2.2.3 Viral infection and immunosuppression

The third theory regarding the mechanisms by which viral infection may predispose to bacterial disease is that the function of the host immune system itself might be impaired by viral infection. This would lead to a suboptimal host defence response once the meningococcus had invaded.

There are a number of different mechanisms by which viruses may cause host immunosuppression.

3.2.2.3.1 Immunosuppression resulting from direct effects on lymphocyte function

Many viruses are lymphotropic and as a result of their replication they are able to impair lymphocyte function. This impairment can involve all classes of

lymphocytes, but more commonly involves subsets of cells such as is seen with Influenza and Epstein Barr viruses (the key viruses of interest in this study).

Epstein Barr Virus is one of the group of Herpes viruses and infection with several members of this viral group has been associated with immunosuppression [^{47;48}]. EBV selectively infects B-lymphocytes resulting in polyclonal hyperimmunoglobulinaemia and the appearance of unusual types of antibodies [⁴⁸]. This results in a transient immune deficiency during the acute illness. In addition, the early B cell lymphocytosis stimulates proliferating T cells, some of which are of the suppressor T cell population. This can lead to a continuing immunosuppressive effect. Abnormal immune function can persist in infected individuals for many weeks [⁴⁸].

It appears that influenza A also has an effect on cellular immunity [⁴⁹]. Although the activity of Natural Killer (NK) cells is enhanced as an early host defence against viral infection, many of the other parameters of cellular immunity are suppressed. Reductions in the absolute number of T lymphocytes and peripheral blood mononuclear cells are seen. This may be clinically significant in determining the outcome of influenza infection [⁴⁹].

3.2.2.3.2 Immunosuppression mediated by suppressive soluble factors

A variety of soluble factors of host or viral origin are released from viral-infected cells. An example of this is the release of interferon from host cells, a cellular protein responsible for mediating anti-viral effects. Interferon induces a wide range of immunoregulatory activities including suppression of lymphocyte function. In vitro, interferon, especially interferon gamma, has been shown to inhibit both cell-mediated immunity and humoral responses although it remains unclear if this occurs at the lower concentrations produced during viral infection. It does however seem likely that interferon changes the functional activity of the various sub-populations of cells involved in immune responses and is the mediator of at least temporary immunosuppression during the early stages of viral infection (reviewed in [⁴⁷]).

Although it is recognised that interferon can cause a range of changes in cells of the immune system, such as altering membrane phospholipid, enhancing cell surface

antigens and altering membrane charge and ion transport, the precise mechanism of exertion of immunosuppressive effects is still largely unknown.

3.2.2.3.3 Immunosuppression resulting from changes in macrophage function

Macrophages are important early defenders against viral invasion. As well as undertaking phagocytosis of viral particles they are involved in lysis of viral-infected cells, mediate antibody-dependant cell killing and exhibit intrinsic and extrinsic anti-viral properties. Furthermore, they play a central role in regulating anti-viral immune responses, stimulating the activity of T cells, B cells and suppressor cells [⁴⁷].

The influence of viral infection on these functions has been little studied. Most widely investigated is the effect of viral infection on the phagocytic function of macrophages. Any of the different phases of phagocytosis may be differentially impaired, resulting in decreased natural immunity and an increased susceptibility to intercurrent infection.

Influenza virus is able to suppress the chemotactic ability of macrophages as well as interfering with the process of fusion with lysosomes once phagocytosis of an organism has occurred. This results in inadequate destruction and hence a concomitant prolongation of intracellular survival of phagocytosed bacteria [⁴⁷].

Viral infection can also impair the antigen processing and presenting abilities of macrophages. It also appears to interfere with the secretion of immunoregulatory molecules such as interleukin-1 (reviewed in [⁴⁷]). However the precise effects of this on immune functioning during common human viral infections is not known.

What does seem clear is that virus-mediated inhibition of macrophage activity is a real phenomenon and can result in a decrease in the host's primary non-specific immune response along with a more insidious decline in the generation of specific immunity.

3.2.2.3.4 Immunosuppression and suppressor cell regulation

The normal response of the host to a foreign antigen involves the complex interplay of cells, some regulating the induction of an immune response and others suppressing it. The result of this interaction determines the nature of the immune response generated and hence the immune status.

The precise activity of these two opposing cellular “armies” is dependent, among other factors, upon the genetic make-up of the host alongside physical properties of the antigen and its route of entry. Infection by certain viruses can change this precise immunoregulatory balance to produce suppressor cell dominance and hence a situation of suppressed immunity.

Most of the work surrounding this theory has been done using the murine model. Evidence suggests that in many cases suppressor cells act as protectors against immunopathological reactions rather than leading to increased morbidity. However the effect of suppressor cell dominance on secondary bacterial invasion is unclear [47].

3.2.2.3.5 Depression of neutrophil function induced by viruses

The polymorphonuclear leukocyte (PMNL) is not highly important in the body's defence against viral invasion but it is the principal cell involved in defence against bacteria and fungi. These are generally the first cells to be recruited to the site of bacterial invasion.

When a PMNL is stimulated a series of events is initiated that begins with chemotaxis (movement of the cell to the site of infection). This is followed by phagocytosis (uptake of the microbe into the cell) and subsequent secretion of lysosomal enzymes (secretory phase) and oxygen products (oxidative phase) to facilitate microbe destruction. Some viruses are known to depress one or more of these functions [50] thereby limiting the effectiveness of the host defence against bacterial invasion.

In addition to these qualitative defects induced by viruses, quantitative deficiencies can also occur in the virally infected patient. This too leaves the host vulnerable to bacterial and fungal infection.

The virus that has been most widely studied in this context is influenza A. It is hypothesised that humans infected with influenza A are at increased risk of infection with *Neisseria meningitidis* [29;31] as well as other bacterial infections [27;51] and it appears that influenza exerts an immunosuppressive effect on PMNL's. However the precise phase of cellular functioning that is affected remains unclear. Neutropenia has been observed three to seven days after influenza infection, a time frame that coincides with the usual time that secondary bacterial infection develops, although neutrophils are not major first-line defence elements against meningococci in particular. Several authors have reported depressed chemotactic activity [52;53] as well as abnormal phagocytosis [52].

It is interesting to note that children with RSV infection have also been reported to exhibit depressed chemotactic, bacteriocidal and oxidative PMNL functioning, although the clinical implications of this are less clear.

3.2.3 Relevance to adolescents

Although some respiratory viruses, in particular RSV and parainfluenza, have a disproportionately high attack rate amongst infants and very young children [54], most respiratory viruses are ubiquitous in affecting all age groups. As already discussed (see section 2.4), young children under five have the highest rate of meningococcal disease with the teenage population having a second smaller peak in incidence. However, in the developing world, where epidemic meningitis is seen, the older age groups are disproportionately more affected during epidemics [32]. Moore et al found that respiratory infections were associated with a greater risk of meningococcal disease in those patients over 15 years old [32]. They suggested that respiratory viruses might have a greater influence on meningococcal disease rates in adolescents and adults than in children by reducing pre-existing immunity; young children are immunologically susceptible irrespective of the influence of respiratory infection.

In the developed world Epstein Barr Virus is one respiratory virus that displays a predilection for the teenage population [^{39;55;56}]. Transmission almost certainly occurs through kissing and increased intimate contact behaviours that develop during this life phase. At five colleges in Britain, 43% of students were seronegative for EBV on enrolling and seven months later 12% of these seronegative students had seroconverted [⁵⁶]. In addition, it has been shown that the percentage of young people seronegative for EBV declines rapidly between the ages of 17 and 22 years [⁵⁶], indicating a high attack rate for this virus in the adolescent and young adult population. Given these statistics and the virus' documented immunosuppressive effects, it seems somewhat surprising that its possible role in the aetiology of the teenage meningococcal disease peak has not been previously investigated.

3.3 Mannose-binding lectin and meningococcal disease

Mannose-binding lectin (MBL) is a calcium-dependant protein belonging to a group of proteins or collectins with similar structures and functions. It is found in the serum of all mammals and appears to play an important role in first-line host defence against both viral and bacterial infection [^{57;58}]. Its role in the aetiology of meningococcal disease has been under investigation for the past decade, with the bulk of clinical research being performed in young children [⁵⁹⁻⁶²]. More recently, with an increasing incidence of fatal disease in teenagers, the possibility of its importance in adolescent disease has been raised.

3.3.1 Pathophysiology and proposed mechanisms of action

3.3.1.1 Structure and function

MBL is able to act directly as an opsonin [⁵⁹] and bind to collectin receptors expressed on various cells involved in cell killing. Hartshorn et al demonstrated MBL acting as an opsonin and enhancing neutrophil reactivity against influenza A [⁶³]. In addition MBL is able to activate the classical complement pathway. MBL can recognise mannose and other related molecules contained within the cell wall of various pathogenic bacteria. These include gram-positive bacteria such as streptococcus and Gram-negative bacteria such as *Neisseria meningitidis* [⁶⁴]. The structure of MBL resembles that of C1q (an initial component of the complement

pathway) leading to an ability to promote the formation of C1 esterase and cleave C2 and C4. This activates the pathway promoting bacterial or viral destruction [^{57;58;64}].

3.3.1.2 Genetics

MBL deficiency is common and may be found in as many as 30% of the population at large [⁶⁵]. Serum concentrations of MBL are genetically determined via three structural genes and three promoter region polymorphisms [⁵⁸]. Low levels of MBL protein can be explained by three point mutations in exon 1 of the MBL gene within codons 52, 54 and 57. The level of serum MBL in the heterozygote depends on which codon is affected, but homozygous individuals usually have undetectable levels. Heterozygotes for the codon 54 or 57 mutations have approximately 1/8 of the wild type level while codon 52 mutants have about 1/2 of wild-type serum concentrations [⁶⁵]. While codon 54 and 57 heterozygosity are the most common mutations, normal populations appear to be relatively depleted of codon 54 homozygotes and this has been postulated to be an effect of early miscarriage of affected fetuses or serious infection early in life [⁶⁵]. The presence of promoter region polymorphisms contributes to the wide range of MBL concentrations observed in all individuals.

It is therefore theoretically possible that a deficiency of MBL could have a clinically important effect on the incidence of or susceptibility to meningococcal disease.

3.3.2 Existing evidence for the role of MBL deficiency in meningococcal disease

In 1968 an infant with severe recurrent infections, chronic diarrhoea and failure to thrive was found to have a defect in opsonisation of baker's yeast [⁶⁶]. Subsequently Super et al defined this specific defect as being that of a deficiency of mannose-binding lectin [⁵⁹] and hypothesised that such a deficiency might be contributory to recurrent or severe childhood infections.

In an attempt to clarify this issue, Summerfield et al studied a series of children aged between 0 and 18 years attending hospital [⁶²]. They found that the prevalence of mutations in the MBL gene in children presenting with an infection was about twice that of those presenting with other non-infectious conditions. This included both the

heterozygote and homozygote states. Of the children who were homozygous for variant alleles a significantly large proportion had severe infections, several of which were meningococcal disease.

Variation in host immunity must be important in determining susceptibility to meningococcal disease, as so many individuals remain asymptomatic carriers of the organism and only a small minority go on to develop invasive disease. In the first six to 18 months of life, as effective antibodies are still developing, innate immune responses are likely to be important [67]. As the peak incidence of meningococcal disease in the UK occurs at around six months of age (see section 2.4.1), this led people to postulate that MBL deficiency might explain why some individuals had a greater susceptibility to meningococcal disease. In the late 1990's, Hibberd and colleagues examined a series of 194 children aged between two months and 16 years admitted to hospital with meningococcal disease. They compared these patients with 272 controls admitted with illnesses other than those related to infections and 110 community controls with no family or personal history of severe infections [68]. They found a significantly increased frequency of both homozygous and heterozygous MBL variant mutations in the patients with meningococcal disease compared with controls. The authors concluded that, based on these results, the proportion of all cases of meningococcal disease occurring in the UK attributable to an MBL deficiency variant was likely to be about a third.

3.3.3 Relevance to adolescents

It was initially thought that MBL deficiency exerted a clinically important effect only on small children between the ages of two and 18 months in whom IgG had not fully developed [65]. However subsequently older children and adults were identified who had the typical phenotype of an immunodeficiency and in whom MBL deficiency was the only identifiable defect [59;62;68]. This indicated that MBL deficiency might confer a lifelong risk of infection and that it could play a role in adolescent susceptibility to infection. It could be argued that the lack of antibody alone could explain infant disease, but as antibody has fully developed by the teenage years MBL might then become the critical factor. Subsequent to the commencement of this study, a study conducted in Denmark looking at adult patients with infection failed to demonstrate a relationship between MBL deficiency and infection and actually

documented higher levels of MBL in the case population than the controls [69]. However, the criteria the authors used to define MBL deficiency was MBL at an undetectable level, meaning that interpretation of these findings warrants caution. However, a similar study published during the period of this data collection that used a Norwegian cohort of patients aged 12 to 21 years, also failed to demonstrate conclusively the relationship between low MBL levels and invasive meningococcal disease [70]. As the mean age of patients in their study was higher than in Hibberd's et al, they suggested that MBL might have an immunoprotective effect chiefly in younger patients and that it might be less important in older teenagers and adults.

To date there are no published data looking at MBL genotype, as opposed to MBL levels alone, in an adolescent population susceptible to meningococcal disease.

3.4 Summary

The observation that infection with a viral agent could predispose an individual to subsequent infection with a bacterium was first described a century ago. With the increasing sophistication of isolation and identification of micro-organisms over the past 100 years, this relationship has now been clarified. Several viruses are now widely accepted as being immunosuppressive while others may predispose to bacterial invasion in a variety of other ways.

Respiratory viral infections are overwhelmingly common in humans. Of this group of viruses, influenza has been the most studied in relation to subsequent bacterial invasion. Following observations that cases of invasive meningococcal disease increased during influenza epidemics, a relationship between these two organisms has been reported. Any link between meningococcal disease and other common respiratory viruses has been investigated but the relationship is less clear.

Adolescents are as vulnerable to these common respiratory infections as other members of the population. In the developed world they are also at a relatively high risk of acquiring Epstein Barr infection. This virus is immunosuppressive and may leave the individual susceptible to bacterial secondary infection, and possibly therefore to meningococcal disease.

Mannose-binding lectin is a relatively recently identified member of the host first-line defence against microbiological attack. Serum levels are genetically controlled and a deficiency of MBL is a common finding among the human population. In children, the relationship between a low serum MBL and the development of frequent or severe infection is a well-established finding. The infections seen are wide-ranging and include infection with *Neisseria meningitidis*.

The effect of low levels of MBL in the adult population is less clear. It seems likely that at some point between childhood and adulthood the influence of MBL levels on the incidence of bacterial infection is less. It is unclear at present whether adolescents with a genetic propensity to low serum levels of MBL still remain at risk.

3.5 Aims of this study

In this chapter possible links between preceding respiratory viral infection and a genetic propensity to low serum MBL levels and invasive meningococcal disease have been explored.

Several risk factor studies have suggested a link between non-specific upper respiratory tract infection and subsequent meningococcal disease [^{41;42}], while others have been unable to find such an association [⁴³].

Adolescents are at particular risk from at least one specific viral infection (EBV) and at general risk from others. In addition they engage in activities that promote viral spread such as increased intimate contact and social mixing. As already discussed, adolescents have an unexplained higher than expected incidence of meningococcal disease. The effect of preceding viral infection on the risk of meningococcal disease specifically in this age group has not been previously studied.

Serum levels of MBL are genetically controlled. While studies on the effect of low MBL levels in children appear conclusive, the effect at the child to adult transition is not known.

In this study the presence of the viruses that might be important in the development of meningococcal disease in adolescence are investigated. In addition, the genetics of three particular MBL mutations in a large population of adolescents will be

examined, and its influence on the development of meningococcal disease in this age group ascertained.

4 Social and behavioural factors and predisposition to meningococcal disease

4.1 Introduction

Adolescence is a period when an individual experiences a complex interplay of social, psychological and biological changes that make this developing adult vulnerable. Experimentation with illicit and licit drugs is a common part of this period of development. Apart from the well- publicised risks of cigarette smoking, the complications of alcohol addiction, or the feared spiral into drug-dependence there may be other effects of such behaviours.

Physical intimacy behaviours usually start in the teenage years as sexual exploration begins. Young adults may move away from home and live in close proximity to other young people, either in a new relationship or during periods of academic study when students often share accommodation.

Elite athleticism has been associated with immune depression and increased risk of infection [⁷¹⁻⁷³]. Other social factors such as low socioeconomic status or a history of breast feeding in infancy have been associated with differing risks of infection in previous studies [^{42;43;74;75}].

The effects of nicotine, alcohol and other drugs on the immune system and other aspects of host defence mean that these social behaviours, often beginning in the teenage years, may form part of the pattern of risk for infectious disease such as meningococcal disease. Similarly as *Neisseria meningitidis* is transmitted via the oropharyngeal route, close proximity and intimacy behaviours may also influence the risk of disease.

In this chapter social factors are considered that operate during adolescent years and that may contribute towards the risk of meningococcal disease and increase our understanding of the teenage peak in incidence of disease. The effect of active and passive smoking will be examined along with other risk-taking behaviours such as alcohol consumption and illicit drug use. Social crowding and intimacy behaviours,

the effect of exercise and the influence of other factors such as socioeconomic status and birth history will be considered.

Existing evidence will be discussed along with possible pathophysiological mechanisms of action. The relevance of these factors to the adolescent population will also be outlined.

4.2 Cigarette smoking and meningococcal disease

4.2.1 Review of the evidence

The relationship between active smoking, passive smoking (exposure to smoke and/or smokers) and meningococcal disease has been studied, particularly for *Neisseria meningitidis* carriage but also for the development of invasive disease.

4.2.1.1 Smoking and carriage of *Neisseria meningitidis*

Carriage of *Neisseria meningitidis* is a prerequisite for disease. The nasopharynx must be colonised, however briefly, before microbiological invasion and disease can occur. Carriage rates are low in young children and reach a peak of almost 25% in the 15 to 19 year age group [¹⁶].

In September 1989, Stuart et al published the results of a case-control study to assess independent risk factors for meningococcal carriage [⁷⁶]. This showed an association between cigarette smoking and meningococcal colonisation of the nasopharynx that was independent of age, sex or social class. In subjects over the age of 12 years, smokers were more than twice as likely to be carriers than were controls. There was also a trend for increased risk of carriage with heavier smoking. In addition, carriers were more likely than controls to live in a household with smokers and therefore to be exposed to smoke and smokers at home.

Other studies have confirmed this association [^{77;78}]. Following an increase in notifications for invasive meningococcal disease in Greece between 1988 and 1990, the factors predisposing to carriage in 1,000 Greek military recruits were studied. Smoking was the factor most strongly associated with carriage in this population with a dose-response effect being clearly demonstrated [⁷⁷]. The results of a similar study, this time involving American jail inmates, were consistent with these findings.

Active smoking increased the inmates' risk of carriage by over 5 times, while passive exposure at home in those inmates just entering confinement more than doubled their risk of carriage [78]. However, a study performed amongst Israeli military personnel at the end of their period of service failed to reach similar conclusions [79]. Although active smoking appeared to be a risk factor for carriage at the univariate analysis level, the association disappeared during multivariate analysis. Passive smoking was not investigated in this population.

Marine commando recruits in Britain were studied shortly after enrollment [80] and in this study both active and passive smoking were significantly associated with carriage. Again, the association between active smoking and carriage was dose-dependant with heavy smokers exhibiting a seven-fold increase in risk of carriage.

Other studies originating from the UK have demonstrated varying results. Blackwell et al studied 400 pupils of a school in Lanarkshire and confirmed a relationship between active smoking and carriage, but no association between passive smoking and carriage [81]. Conversely, Fitzpatrick reported passive smoking as being important for carriage amongst a population of schoolchildren whilst active smoking was significant only at the univariate level of analysis [82]. Finally a carriage study in rural Wales failed to show that either passive or active smoking were associated with carriage in the multivariate analysis, despite showing active smoking to be related at univariate level [83]. However, this study was relatively small and may have been underpowered to show the association suggested by the univariate results.

It seems likely that a relationship exists between smoke/smoker exposure and meningococcal disease. However the precise nature of smoke exposure (active versus passive) and/or exposure to smokers themselves that is conferring risk seems unclear. The inherent difficulties of measuring or quantifying passive smoke exposure and exposure to smokers may be confounding many of these studies.

4.2.1.2 Smoking and invasive disease

Studies suggest that, as well as leading to increased carriage rates, tobacco smoke exposure might also increase the risk of developing invasive meningococcal disease. Work from Norway, Britain, Australia and the United States published since 1983

suggests passive smoke exposure in children may be of particular importance in conferring risk of disease [^{43;44;75;84}].

Probably the earliest study addressing this issue came from Norway and showed that passive smoke exposure in children under the age of 12 years was associated with disease [⁴⁴]. The proxy used for passive smoke exposure was a member of the household who smoked. Active smoking among the case adults was not related to disease. There was a suggestion that mortality amongst children exposed to passive smoke was greater than those not exposed, although the result did not reach statistical significance. Subsequently a large case-control study investigating risk factors for the development of meningococcal disease in Britain established a strong epidemiological link between meningococcal disease in children under 5 years old and exposure to tobacco smoke [⁴³]. Results showed that almost twice as many cases as controls were exposed to moderate or heavy passive smoke loads at home. A dose-response relationship was observed both with number of smokers in the household and number of cigarettes smoked by each. Again mortality was greater in the cases exposed than those not, although these results also did not reach significance.

A study using a similar design and performed in the USA reproduced these results but for children under the age of 18 [⁷⁵]. They found that the strongest independent risk factor for the development of invasive meningococcal disease for all children and teenagers under the age of 18 years was having a mother who smoked. The relationship was strongest for the subset of cases under the age of five. They were also able to demonstrate a dose-response relationship between risk of disease and number of packs smoked per day. The group concluded that 37% of all cases of meningococcal disease in people of this age could be directly attributable to maternal smoking. In addition, they demonstrated a relationship between both active and passive smoking in the adult cases and meningococcal disease. More recently, these findings were replicated by an Australian team who identified young children with a primary caregiver who smoked as being at increased risk of disease although this study did suffer from methodological issues in control selection and matching and from relatively small numbers. [⁸⁴].

Further studies from the USA and Czech Republic confirmed the relationship between passive smoke and/or smoker exposure in young children and invasive

meningococcal disease [74;85]. A single risk factor study from Cape Town failed to find an association, but this was probably due to the high rate of smoking in both case and control households [42].

Adolescence is a period of time when adult smoking habits are often formed and when passive exposure to smoke increases through changing social behavioural patterns (frequenting bars and clubs and developing closer relationships with other smokers). Smoke exposure may therefore form one of the important risk factors for disease acquisition in this population. As previously stated there are to date no large risk factor studies with adolescents as subjects. The American study of college students by Bruce et al [41] looked only at active smoking, and while there was the suggestion of an effect on univariate modelling, once the multivariate analysis was complete this effect disappeared suggesting the effect was due to confounding by other social variables.

4.2.2 Pathophysiology and proposed mechanisms of action

There are plausible mechanisms to explain the relationship between cigarette smoke exposure and both increased carriage of meningococcus and invasive disease. Cigarette smoke inhibits mucociliary clearance, enhances bacterial adherence to cells of the nasopharynx and disrupts the respiratory epithelium [86-88]. As the primary site of acquisition of the bacteria is the nasopharynx, it seems possible that damage in this area might lead to difficulties in clearance of invading bacteria. Furthermore tobacco smoke has been shown to impair neutrophil migration and inhibit the phagocytic activity of macrophages, primarily in the respiratory tract [89]. In vitro experiments have demonstrated suppression of immunoglobulin production [86;87]. Such effects may promote invasion of the meningococcus.

It is also known that smokers and young children exposed to smoke in the home have an increased incidence of viral respiratory tract infection [90]. As discussed in section 3.2.1 there is a possible relationship between meningococcal disease and respiratory viral infection. It may be that smoke exposure and respiratory viral infection might act as co-factors to increase risk of disease.

4.2.3 Relevance to adolescence

Experimentation with cigarette smoking is common during adolescence [⁹¹]. In the late 1980's in Great Britain 450 children started smoking every day and by the age of 15 years a quarter were smoking regularly [⁹²]. The vast majority of adult smokers have established their habit by the age of 18 as most new smoking recruits are under this age [⁹²]. A large study published in 1994 that analysed data collected in secondary schools in Doncaster, Northern England, showed that almost 1 in 4 girls and 1 in 6 boys aged between 15 and 16 years were regular smokers [⁹³]. Although there had been tentative hopeful reports that teenage smoking was on the decline [⁹⁴], this study reported an overall increase in prevalence of 8% since a similar survey carried out in 1988.

An interesting cohort study by Stanton et al [⁹⁴] followed the health, development and behaviour of over a thousand young people between the ages of 3 and 18 years. Their smoking-related behaviours were studied from the age of 9 years onwards. Part of the analysis concentrated on the period of time between the ages of 15 and 18, and the results showed a dramatic increase in the prevalence of daily smoking from age 15 (15%) to 18 (31%). The majority of 18-year old daily smokers indicated that they first became regular smokers between the ages of 15 and 17 years. Although this study was performed in New Zealand, work in the U.K has indicated a similar pattern [^{91;95}].

The average number of cigarettes smoked per week in the Doncaster cohort was 39 per person, a number compatible with significant physiological effects and withdrawal symptoms on cessation [⁹³]. A trend for increasing number of cigarettes smoked per day was also found in a younger group of teenagers in South-East England between 1975 and 1979 [⁹⁵].

A common criticism of any study that relies on self-reporting, especially in a population of teenagers, is that the validity of such results is questionable. This has led to the suggestion that any such data should include a biological measure for validity comparison. Williams et al however found a poor correlation between both objective measures and a prior warning system and smoking behaviour, and suggested that adolescents reported truthfully about smoking behaviours when faced

with anonymous questionnaires [96]. In general it is felt that teenagers report accurately on their smoking behaviour patterns, although their recall ability for periods of time beyond one year is unsatisfactory.

4.3 Alcohol, recreational drugs and meningococcal disease

4.3.1 Alcohol and meningococcal disease

For over 200 years the potential for excess alcohol consumption to adversely affect health in relation to infectious disease has been recognised. In 1785 Benjamin Rush wrote a piece entitled “ An enquiry Into the Effects of Ardent Spirits Upon the Human Body and Mind” in which tuberculosis, yellow fever and pneumonia were all listed as complications of imbibing the “ardent spirits” [97]. In the late 19th century experiments began on intoxicated rats which were noted to be more susceptible to cholera than their sober cage-mates. Retrospective observational studies in humans continued to document the predisposition to infectious disease, in particular to tuberculosis and some opportunistic infections, in alcohol abusers throughout the early 20th century. However these studies were uncontrolled and conclusions drawn subject to a wealth of confounding factors. More recently the effect of alcohol on the immune system has been subjected to the more formal rigours of modern research technique.

4.3.1.1 Review of the evidence

In early 1991 in North America there was an outbreak of serogroup C meningococcal disease amongst a group of college students between 18 and 22 years of age. This outbreak provided the opportunity to study predisposing factors in this population. The results of this study showed that carriage rates for *Neisseria meningitidis* were five times higher in those reporting having drunk alcohol in the preceding week than in those who hadn't. A dose-response effect was also observed [98]. The authors commented that this was the first reported association between meningococcal carriage and alcohol consumption, but admitted the difficulty with possible confounders (crowded, smoky and noisy environment of bars and pubs, intimacy behaviours promoted etc). Although multiple logistic regression analysis did produce alcohol consumption as an independent variable for meningococcal carriage, data suggested patronage of a particular bar to be more important than actual alcohol

consumed suggesting environmental features of the bar or characteristics of the attendants at that bar to be confounding the results. Haneberg et al [⁴⁴] noted a high mortality rate in heavy drinkers contracting meningococcal disease but found no significant correlation between alcohol consumption and disease occurrence. A small outbreak study from Argentina found attendance at a particular disco to be a risk factor for development of disease, although alcohol consumption per se was not implicated [⁹⁹]. A very similar study from Sydney, Australia also found attendance at a nightclub to be linked to disease status, but again alcohol consumption itself was not measured [¹⁰⁰]. These studies are almost certainly identifying other behavioural risk factors associated with nightclub attendance. In a study using jail inmates as subjects to investigate risk factors for meningococcal carriage, those individuals charged with alcohol or drug-related crimes were one and a half times more likely to carry serogroup C meningococcus than those charged with traffic-violation offences or jaywalking [⁷⁸]. However this result did not reach statistical significance. More recently a large, well-conducted study from the United States recruiting 18 to 22 year-old college students as subjects, found no association between alcohol consumption and meningococcal disease on multivariate analysis, although they did find it to be a risk factor during univariate analysis [⁴¹].

These findings are inconclusive at best. It seems reasonable to conclude that alcohol consumption may simply be a marker for other high-risk behaviours such as crowding, intimate contact or exposure to active or passive smoke or smokers. However, there are pathophysiological reasons why drinking alcohol might predispose to infectious disease, a summary of which follows.

4.3.1.2 Pathophysiology and possible mechanisms of action

Alcohol abuse impacts on many areas of society. One of these areas is that of personal health. Chronic alcoholics have an increased incidence of cirrhosis of the liver, gastrointestinal haemorrhage, trauma, cancer and infectious disease, in particular tuberculosis, bacterial pneumonias and opportunistic infections [¹⁰¹]. Malnutrition is a common finding in the alcoholic patient and this is known to adversely affect immunity to infection. However it has long been believed that alcohol itself interferes with immune function in a clinically significant manner.

A number of studies summarised by MacGregor [¹⁰¹] focus on the specific effects of alcohol on various modalities of the immune system. Chronic drinking significantly depresses myeloid reserves leading to neutropenia in response to infection as well as preventing normal delivery of polymorphonuclear neutrophils to sites of bacterial infection. Acute intoxication inhibited these cells' adherence to endothelial cells necessary for subsequent migration into tissue sites. Such effects were independent of alcoholic liver disease.

Alcohol consumption also may have an effect on cell-mediated immunity as suggested by the increased incidence of tuberculosis and opportunistic infections observed amongst alcoholics [¹⁰²]. Control of such infections is dependent on effective cell-mediated immunity. In vitro studies have demonstrated interference with cell-mediated immune function, in particular with NK cells. Ben-Eliyahu et al [¹⁰³] demonstrated marked suppression of NK cells in rats in vivo associated with ethanol ingestion.

MacGregor also reports suppression of macrophage function leading to inhibition of particle clearance by the lungs, liver and peritoneum as well as the interference with antibody responses to new antigens and the loss of bactericidal activity resulting from alcohol ingestion, both chronically and acutely [¹⁰¹].

It is unclear at present to what extent the effects on immune function observed in vitro have clinical significance. Macgregor concludes his review by stating that any individual using alcohol in excess should be considered immunosuppressed, but there is little evidence that this can be extended to social drinkers. In 1994 Bounds et al published work looking specifically at the effects of alcohol ingestion in a social context on immune functioning [¹⁰⁴]. This was a case-control study using small numbers of subjects who consumed approximately a pint and a half of beer whilst eating pizza. They found no effect on NK cells but demonstrated impairment of the cytotoxic capacity of peripheral blood mononuclear cells. They were however hesitant to suggest that this may have a clinically significant effect in otherwise healthy individuals.

4.3.1.3 Relevance to adolescence

Just as teenagers experiment with cigarettes, drinking alcohol is often one of the earliest forms of experimentation in the adolescent years. Although recent evidence suggests that a substantial minority of adolescents drink heavily [¹⁰⁵], it is likely that a substantial majority drink in some context. In 1996 a large international survey examined patterns of self-reported drinking behaviour amongst over 7,000 15 and 16 year-olds in Europe [¹⁰⁶]. For the English teenagers in this study, the results confirmed the suspected frequency of alcohol consumption. Nearly 95% of subjects reported ever having consumed alcohol, with between 75 and 80% reporting intoxication at some point. The mean intake of alcohol on the last occasion that a study subject drank was 6.5 units for girls and 8.5 units for boys. The study concluded that the overall frequency of drinking amongst this age group had increased over the previous five years.

4.3.2 Illicit drug use and meningococcal disease

4.3.2.1 Review of the evidence

To date there are no studies specifically examining the effect of illicit drug use on the risk of developing invasive meningococcal disease. A single American study using jail inmates as subjects noted that those charged with drug-related offences were one and a half times more likely to develop meningococcal disease than those charged with other minor offences [⁷⁸]. This result did not reach significance and must be subject to a great deal of confounding.

4.3.2.2 Pathophysiology and possible mechanisms of action

The effect of illicit drugs on the immune system is largely unclear although it has been suggested that these drugs may affect the resistance of nasopharyngeal mucosal cells to colonisation with meningococcus in a manner comparable to viral infection or cigarette smoking [⁷⁸]. There is some in vitro evidence suggesting that marijuana may modulate the immune system and suppress host resistance to infection [¹⁰⁷] but the clinical significance of this is unclear.

4.3.2.3 Relevance to adolescents

Measurement of drug-using behaviour amongst adolescents is complicated by the fact that it is illegal and therefore subject to reporting bias. However, data suggest that it is common. In a large survey of teenagers published in 1996, 40% of 15 and 16 year-olds reported ever having taken an illicit drug. The majority had taken cannabis, with 20% having inhaled glues and solvents and nearly 10% having tried ecstasy – a category A drug [¹⁰⁶]. Not surprisingly the authors found a strong relationship between cigarette smoking and cannabis use.

4.4 Overcrowding, intimate contact and meningococcal disease

In 1918, Glover published the results of a study that became the foundation stone for theories relating meningococcal carriage and disease to crowded living conditions despite some major methodological and reporting problems [¹⁰⁸]. Military barracks were the setting for this study and a relationship between the more crowded barracks and a higher incidence of meningococcal disease was confirmed. It was also found that a reduction in risk could be achieved by increasing the distance between beds in the barracks.

Further work to investigate the relationship between household overcrowding and other forms of close contact living and risk of meningococcal disease has subsequently been carried out. In the following review two broad categories of close contact living are considered. The first relates to household overcrowding and the second to other forms of close contact outside the home such as social intimacy behaviours and crowding within schools or colleges and other social environments relevant to the adolescent.

4.4.1 Review of the evidence

4.4.1.1 Household overcrowding

In order to analyse the effect of overcrowding on health, it is first necessary to define the term. In the slums found commonly during Queen Victoria's reign, the number of people sleeping in each room was a useful indicator of the degree of crowding [¹⁰⁹]. With today's modern and spacious living it is more difficult to define. The Registrar General's annual reports define overcrowding as more than two people per

habitable room (all rooms excluding bathroom and kitchen) [¹⁰⁹]. Studies examining the effects of crowding on risk of meningococcal disease have employed varying definitions. Some use surface area available for living [¹¹⁰], some use number of persons sharing a bedroom [⁷⁸] whilst others use the number of people per habitable room [^{42;43}].

It is commonly believed that overcrowding is more of a threat to mental than physical health [¹⁰⁹]. If the number of people using a dwelling is separated from other associated variables such as social class and poverty it has very little influence on health [¹⁰⁹]. However, invasive meningococcal disease rates are related to carriage rates and household contacts exposed to a case of meningococcal disease have a 500 to 1000-fold increased risk of disease compared to the general population [¹¹¹]. It seems intuitive that close contact within overcrowded living conditions may increase the opportunities for transmission of an organism that is carried within the nasopharynx.

4.4.1.1.1 Household overcrowding and carriage of meningococcus

Several studies have examined the effect of overcrowding on meningococcal carriage rates as part of a more comprehensive risk factor investigation [^{78;79;82}]. Two of these studies linked shared living accommodation within a jail [⁷⁸] and a military barrack [⁷⁹] to increased carriage, although for the jail inmates it was overcrowding (>two people per bedroom) prior to booking that conferred increased risk. The study performed within the military barracks did not define overcrowding, using only service at a closed rather than open base to indicate personal overcrowding. A third study found no association between a household density ratio (ratio of number of household members to number of rooms) to carriage rates [⁸²]. Comparisons between these studies are difficult due to the differing measures of overcrowding employed, but it has been suggested that household overcrowding needs to be extreme in order to influence carriage rates [^{83;112}].

4.4.1.1.2 Household overcrowding and invasive disease

Other studies have looked more specifically at rates of invasive meningococcal disease in relation to overcrowding. Again results are inconsistent. Two large

studies performed in the UK were able to directly or ecologically demonstrate a relationship between invasive disease and overcrowding [^{43;113}] as was a large study from South Africa [⁴²]. However the strongest association in all these studies was for young children under five years of age, and again precise definitions of what constitutes overcrowding varied. In a further study from the United States children who lived with other children in the household were found to be at increased risk for meningococcal disease, but overcrowding itself was not examined [⁷⁵]. In a recent Australian study children under the age of six years who shared a bedroom with two or more people were found to be at greatly increased risk of disease, and this included babies sharing a room with parents [⁸⁴]. It is important to bear in mind socioeconomic status when critically evaluating studies examining the effects of overcrowding. It is likely that poverty and low socioeconomic status have a greater adverse effect on health than overcrowding alone [¹⁰⁹], although it can be hard to separate the two. In the studies by Stanwell-Smith et al and Jones et al, overcrowding remained an independent risk factor once socioeconomic status was controlled for although residual confounding may still exist [^{43;113}].

Not all studies have confirmed this relationship. In a small, and probably underpowered study, involving school children from the USA, there was no difference between cases and controls in terms of household size or crowding using the crowding index (number of occupants per number of rooms) [¹¹⁴]. A second study conducted in The Gambia, West Africa included large numbers of affected individuals and again there was no relationship between number per bedroom or mean sleeping area per person and incidence of disease [¹¹⁰].

4.4.1.2 Intimacy, close personal contact and social crowding

4.4.1.2.1 Social crowding

As well as crowding within the home environment, it is also important to consider other forms of close contact between individuals that may predispose to transmission of the meningococcus. The focus for many of the studies designed to address this issue has been on outbreaks within school environments. In the largest of these studies 22 school-based clusters of meningococcal disease throughout 15 states in America were examined and an attempt made to analyse the activities of the children

involved [¹¹¹]. In 16 of the 22 clusters, case patients had more direct interaction with each other within the school than unaffected individuals. These interactions included sharing a classroom, riding the same school bus, attending mutual gatherings (field trips, school dances) and participating in the same extracurricular activities. Recall bias may be a problem in this analysis. In other studies the distance between chairs in the classroom, [¹¹⁵], lunchtime contact [¹¹⁵], riding on a particular bus [^{30;116}] and attending various social events [^{83;116}] were identified as being risk factors.

School-based clusters of meningococcal disease are more common amongst high-school students than among younger age groups raising the possibility of secondary school clustering being a marker for behavioural risk factors that may facilitate transmission of the meningococcus in teenagers [¹¹¹]. In a study by Zangwill et al, case patients tended to participate more in school activities than controls, theoretically facilitating exposure.

Another group that is traditionally subject to outbreak situations and have a higher than expected background rate of meningococcal disease is college or university students [¹¹⁷]. In a case-control risk factor study from the United States students living in dormitories were at significantly increased risk of disease, but those sharing a bedroom were not [⁴¹]. Other authors have identified campus living [¹¹⁸] and attendance at universities with a large amount of catered hall accommodation [¹¹⁷] as being risk factors for disease. These indicate that factors associated with close personal contact may increase risk of disease in a student population.

Other social and behavioural elements of teenage life that involve close personal contact have also been examined in some studies. In two small studies clusters of meningococcal disease were reported in association with nightclub attendance [^{99;100}]. Bruce et al, in his population of students, found that frequenting parties or bars was a risk factor at univariate analysis, but non-significant in the multivariate model [⁴¹]. Interestingly this study identified movie attendance to be significantly protective against disease. However movie attendance was inversely correlated with smoking, bar patronage and alcohol consumption, illustrating the subtle and complex interplay of social factors operating.

4.4.1.2.2 Intimate and close personal contacts

As *Neisseria meningitidis* is an organism that colonises the nasopharynx, intimate or deep kissing might facilitate transmission. Surprisingly, little work has been done to address this, perhaps due to the fact that most invasive disease risk factor studies have been performed in children.

Stanwell-Smith et al specifically addressed intimate contact in a large case-control study including children and adults. They found that the number of regular close contacts or cheek kissing contacts was not associated with disease, but that mouth kisses with four or more contacts in a two-week period prior to disease in children under five years old was significantly associated with disease [⁴³]. In a similar study from Australia there was no relationship between number of close contacts of cases and disease risk, but intimate kissing was not addressed [¹¹⁹]. In only one study was intimate contact specifically examined in a young adult population, and kissing more than two people in the month prior to illness was found to be important only during univariate analysis [⁴¹]. This area merits further investigation.

4.5 Exercise and meningococcal disease.

Physical exercise is traditionally believed to be the gateway to good health and longevity. The role of public health promotion programmes in reinforcing these attitudes has been substantial. The positive effects of exercise are indeed multiple and one is led to believe that no amount of exercise can be too much. However, evidence suggests that exercising to the extreme may in some situations be detrimental to health, increasing the susceptibility of an individual to infectious disease.

This theory will be briefly examined here and the relevance to adolescent meningococcal disease investigated.

4.5.1 Review of the evidence

The beneficial effects of moderate amounts of exercise on health are well publicised, as too are the detrimental effects of complete inactivity. However, the balance of risk and benefit of vigorous activity is becoming subject to debate. Evidence suggests

that vigorous, strenuous activity does indeed have a beneficial effect on cardiovascular risk [¹²⁰] but more recently studies have emerged involving elite athletes who undergo rigorous training schedules and appear to become immunosuppressed as a result [^{71;72}]. Such an effect on the immune system may increase the susceptibility of such individuals to infectious disease.

There are several reports of increased incidence and severity of infectious disease amongst sportsmen. A hepatitis outbreak in a US college affected 90 out of 97 members of a football team whilst no other members of the student population were affected.^[121] All nine boys who contracted polio in one school were participating in strenuous sports [¹²²]. In an outbreak of coxsackie B meningitis 63% of football team members were found to be IgM positive compared to 12% of other students [¹²³]. Of course, a key confounder in these studies is the close and prolonged contact between the sportsmen, as well as the possibility of shared exposure to contaminated food or water.

Hughes reviewed the literature concerning mild, moderate and strenuous exercise and the incidence of infection [⁷¹]. He concluded that mild exercise had no effect, either beneficial or detrimental, on the incidence of infection while individuals who participated in moderate amounts of exercise had fewer infections. One study illustrating this point compared groups of elderly women who were sedentary, undertook mild exercise or undertook moderate exercise. The moderately exercising group had an incidence of upper respiratory infection of 8% during the three month observation period, compared to an incidence of 21% in the mildly exercising group and 50% in the sedentary group, suggesting a protective effect of moderate exercise [¹²⁴]. However it is also possible that those individuals able to tolerate more exercise are a healthier group and are more resistant to infection.

Running is deemed to be one of the most taxing forms of exercise, and marathon running provides an ideal opportunity to study the effects on the immune system of such a vigorous activity. In two groups of entrants to the Los Angeles marathon, Nieman et al found the incidence of upper respiratory tract infection in the week following the race was significantly higher in the running group than in those who registered but did not run [¹²⁵]. Reasons for the entrants withdrawing were not however made explicit and may well have included the presence of a viral URTI in

the week preceding the race, precluding participation. A similar study reviewed by Hughes involving the Cape Town marathon found that the incidence of upper respiratory tract infection in runners was twice that of non-runners. The literature suggests a J-shaped relationship between regular exercise and immunity to infection with a decreasing risk of infection from mild through to moderate exercising and an increased risk with prolonged strenuous exercise.

4.5.1.1 The effect of physical activity on the immune response – possible pathophysiological mechanisms

In normal individuals the numbers and activity of lymphocytes, NK cells and lymphokine-activated killer cells increases during a period of acute exercise, after which the levels fall to below normal for a period of about 6 hours [73]. The neutrophil levels remain high. These changes are seen both in athletes and in untrained individuals. When trained and untrained individuals exercised to exhaustion, a reduction in T-lymphocyte helper/ suppressor ratio and in NK cell responses was observed for up to 20 hours [71]. Reductions in salivary IgA concentrations have been observed following strenuous cycling [72]. Other studies reviewed by Fitzgerald [72] have confirmed these effects on immune parameters with vigorous exercise. NK cell activity was significantly reduced for several hours in subjects undergoing a single exhausting exercise session, whereas the effect was not observed in the same subjects undergoing moderate exercise. Marathon runners were noted to have lymphocyte counts of less than 1500; Russian sportsmen had declining levels of serum immunoglobulins, secretory IgA and non-specific immunity as their exercise schedules increased.

Similar effects presumably occur each time an athlete competes or trains hard, such that these individuals are undergoing repeated episodes of temporary immunosuppression on many days of each week. This “open window” theory of post-exercise susceptibility to infection leaves the elite athlete vulnerable to repeated infection.

4.5.1.2 Relevance to adolescents

Adolescents between the ages of 11 and 21 years are the group most commonly involved in strenuous organised competitive sports [71], although exercise prevalence declines steeply between the ages of 15 and 24 years [126]. This suggests that there is

a small group of adolescents older than 15 years that continues to engage in vigorous sporting activity. These individuals may be at increased risk from infectious illness because of temporary immunosuppression occurring as a result of multiple effects on various components of the immune system. However, the close contact experienced between members of a sporting team may serve to increase transmission of infectious agents, demonstrated by a predominance of cutaneous infections amongst athletes [127]. Such athletes also have shared exposures to infectious agents as well as exposure to new organisms when travelling to compete and during socialisation with other teams.

The relationship between development of meningococcal disease and strenuous exercise has not been defined and requires further exploration.

4.6 Other social factors and risk of meningococcal disease

Poverty and low socioeconomic status have long been associated with ill-health. The relationship between low socioeconomic status and invasive meningococcal disease is also well-established [43;74;75;85;128]. However, a variety of different measures have been employed to define socioeconomic status and this can make comparisons between studies difficult. Many of these studies involve children and use the level of maternal education as a predictor of social class [74;75;85]. Stanwell-Smith examined all ages and used the traditional Registrar General's classification of socioeconomic status based on occupation of the head of household. Whilst significant protection from invasive disease in all age groups was afforded by high social class (1 or 2), significant risk was only conferred by being social class 3 and not by being social classes 4 and 5 [43]. This unexpected result may have been due to inadequate powering of the study, although an explanation is not provided within the paper. In a large study of college students there was no link between social class, as measured using household income, and invasive disease [41], although the number of lower social class subjects in a student population is likely to be small. In other more recent studies area based measures of deprivation were employed to examine the issue, and confirmed low socioeconomic status as being a major risk factor for disease especially in the under five year age group [128-131].

There is no specific reason to believe that the adolescent population would be unique or different in terms of social class and meningococcal disease risk. However, the measurement and recording of social class is important as other social variables such as smoking and drug-taking prevalence may differ between socioeconomic groups and this needs to be considered during analysis of results.

Several other social factors have been investigated as part of larger risk factor investigations. The effect of early breast feeding on subjects' later risk of developing invasive meningococcal disease has been examined in several studies. Stanwell-Smith et al found no relationship between type of feeding and disease [⁴³], while McCall reported a protective effect at univariate analysis only [⁸⁴]. A South African risk factor study reported breastfeeding for less than three months as being a risk factor [⁴²]. Conclusions of this study should however be interpreted with caution as the results did not include measurement of, or controlling for, socioeconomic status, a variable strongly associated with breast feeding behaviour.

Over the past decade, much attention has focused on the relationship between birth weight and later adult morbidity. In particular work has focused on cardiovascular morbidity during adulthood and its link with low birth weight [¹³²⁻¹³⁴]. Slightly less importance has been given to the possible relationship between premature birth and later disease risk. A single study from the United States designed to examine the effects of maternal smoking on risk of developing invasive disease in children, recorded birth weight and gestation of subjects who later went on to develop disease [⁷⁴]. This study carefully controlled for social class, and found no significant association between birth weight or premature delivery and later meningococcal disease. However this was an incidental finding in a study designed to examine a different exposure. Premature delivery and low birth weight has been linked to increased infectious disease mortality up to the age of seven years, the main effect being due to prematurity rather than to being small-for-gestational-age [¹³⁵].

Preterm birth has also been associated with an increased risk of hospitalisation with infectious diseases up to the age of five years [¹³⁶]. In only one study was there a suggestion that intra-uterine environment might impact on later immune functioning up to adolescence [¹³⁷], although this study examined small-for-gestational-age babies rather than premature infants. In a single study the issue of prematurity and

low birth weight has been addressed with respect to meningococcal disease. Low birthweight was associated with an increased risk of meningococcal disease throughout childhood but the increased risk of meningococcal disease seen in premature infants was only apparent for the first year of life [138]. This study carefully controlled for maternal smoking behaviour, known to influence birth weight and gestation, in addressing the risk of meningococcal disease during childhood, but did not control for other confounders.

The reasons why prematurity and/or low birth weight might increase later susceptibility to infectious disease are unclear. It is possible that differences in immune programming related to timing of birth may play a role [135;137;138], as might maternal pre- and postnatal lifestyle [138]. Prenatal undernutrition leading to low birth weight and possibly to an increased risk of premature delivery, has been linked to defects in cell-mediated immunity and antibody response to vaccination [137]. Although there seems to be biologically plausible mechanisms for an association, there are no published data on the risk of prematurity and infectious disease morbidity during adolescence.

Finally, in a single risk factor study regular church attendance was identified as being a protective factor against the development of meningococcal disease [75]. Other authors have suggested a protective effect of religious belief for all-cause morbidity [139;140], although a more recent study found no protective effect of intercessory prayer on cardiac outcome in adults [141]. While religious observance might simply act as a marker for other behaviours that reduce risk, it is plausible that the social support gained from a close community with shared belief promotes genuine beneficial effects quite apart from any “power of prayer”.

4.7 Summary

Adolescence is a period of social change. Social networks may increase, households may change, social behaviours alter and intimate relationships develop. The result of such adaptations is a change in exposures that may alter an individual's risk of either meningococcal carriage or disease.

There is an extensive body of literature examining social risk factors on the risk of both carriage and disease. However, the results of some of these studies should be

interpreted with caution and in the light of knowledge concerning sample size, possible biases and confounding variables. Comparisons between studies can be difficult if different measuring tools are employed, such as with passive smoke exposure, socio-economic status and crowding variables. Social and behavioural factors are notoriously subject to confounding, which can be difficult to fully control for and the effects of residual confounding must always be considered. As many of these studies employ case control methodology, the effects of bias, in particular selection and recall bias must be considered during critical analysis of results.

The teenage years are when cigarette smoking experimentation and the formation of lifelong habits generally develop. In addition, due to changing social networks, passive smoke exposure outside the home environment may increase. A comprehensive body of literature exists that defines the relationship between both active and passive smoking and the carriage of, and disease caused by, the meningococcus. Both active and passive smoking appear to increase carriage rates, although the inherent difficulties in measuring and quantifying passive smoke exposure lead to conflicting results. In children passive smoke/smoker exposure within the home may increase the risk of developing invasive meningococcal disease. Plausible pathophysiological mechanisms underlie these findings, with the damaging effect on nasopharyngeal mucosal tissue increasing the likelihood of colonisation and invasion.

Experimentation with other substances also typically begins during this life phase. The number of young teenagers who have not only tried alcohol but have been intoxicated is high. Similarly a substantial minority of young people experiment with all classes of illegal drugs. There is little evidence that alcohol consumption per se increases risk of meningococcal disease – rather it may act as a marker for and a precipitant of other social behaviours occurring simultaneously and conferring risk. Although there are theoretical pathophysiological mechanisms for alcohol increasing infectious disease risk through an effect on immune functioning, the clinical significance of these are unclear, especially with social levels of drinking. There are no published studies investigating the effect of drug use on meningococcal disease risk.

Overcrowded living conditions were probably one of the first identified risk factors for development of invasive meningococcal disease. The most plausible explanation for this was through increased transmission of the causative organism both via normal routes and through increased coughing during outbreaks of respiratory viral infections common in crowded living conditions. Subsequent opinion on this issue is divided. A relationship between overcrowding and both carriage and disease has been demonstrated in some studies but not in others. Problems surrounding this area of study include the measurement and definition of overcrowding and its close relationship with low socioeconomic status and poverty. Household overcrowding levels probably need to be extreme to influence disease, and it is likely that these levels are infrequently seen in the developing world.

Crowding and close contact within other environments may also facilitate transmission of the meningococcus and therefore influence disease rates. Outbreaks traditionally occur within schools and colleges, and associations have been made between shared social activities and disease. Intimate or mouth kissing has also been linked to disease. The crowded atmosphere of bars and nightclubs frequented by young people along with the high prevalence of cigarette smoking in these places, the possibility of intimate liaisons and the need for close face to face shouting to be heard, might explain disease patterns in this age group.

Athletes have an increased incidence of infection, in particular respiratory viral and cutaneous infections. Studies have documented varying effects of extreme athleticism on the immune system leading to a degree of immunosuppression that could possibly be clinically significant. The close contact afforded between members of sporting groups or teams might also serve to increase transmission of causative organisms. A small sub-group of the adolescent population engages in extreme strenuous exercise. A larger proportion are involved in team sports. The relationship between extreme exercise regimes and the development of meningococcal disease has not been investigated.

Finally, the possibility of other social factors that may be of importance to this population and their disease risk were reviewed. Low socioeconomic status is universally associated with an increase in general morbidity and more specifically with an increased risk of developing meningococcal disease. It is an important

variable to record accurately as it has confounding effects on many other variables measured. Early immunological programming may influence later health, and there is a possible relationship between prematurity, early breast-feeding and protection from invasive disease, although this has not been widely investigated or reported. Religious observance may be associated with a lower rate of behavioural risk-taking events.

4.8 Aims of this study

Some areas of social living and behaviour have been comprehensively investigated in relation to meningococcal disease risk whilst others remain largely unexplored. Despite adolescents being a group subject to extreme social adjustment and having a higher than expected incidence of meningococcal disease, they have been little investigated as a group.

This study aims to clarify the relationship between cigarette smoke exposure and disease in teenagers by collecting data relating to both active and passive smoke exposure. The possibility of drug-use and disease will be investigated for the first time, whilst data concerning alcohol intake and social mixing behaviours will be sought. Information about adolescents' social contacts and intimacy behaviours will be collected and related to disease risk, as will their exercise habits.

Although a large number of potential risk factors have been reviewed here, a number of these will be assessed within this study in order to address confounding and not as primary hypotheses.

The aim is to produce a more comprehensive picture of the social behaviours operating during adolescence and their effects on the young individuals' disease risk.

5 Psychological stress, social support networks and susceptibility to infectious disease

5.1 Psychological stress & infectious disease

5.1.1 Historical perspective

The concept that the emotional state of an individual could be linked to their state of health is not new. From before the time of Hippocrates, 500 years B.C, the “passions” were believed to be fundamental players in the causation of disease. The four humors of the body had to remain in balance for health to be maintained and the diseased state was treated by restoration of a supposed imbalance [¹⁴²]. Subsequent eras have brought with them the differing views of “science”, from popular theory to the realms of the magical.

Research on the relationship between stressful life events and the occurrence of disease began in the late 19th century exemplified by Pasteur’s observation that chickens normally resistant to anthrax became susceptible when immersed in cold water [¹⁴³]. In 1936 Selye conceptualised stress with the introduction of the “general adaptation syndrome”. This was noted in animals chronically exposed to noxious stimuli and consisted of a triad of features produced by activation of the pituitary-adrenal axis. Enlargement of the cortex of the adrenal gland, atrophy of the thymus and other lymphoid structures, and development of bleeding ulcers of the stomach and duodenum were all seen. Initially only stressors of a physical nature were considered to be important, but with passing time the notion that psychological stress might also affect bodily functioning took hold.

Modern science has resurrected the interest in a relationship between emotional state and disease. When exposed to an infectious agent only a proportion of people will develop disease. The severity and duration of illness also vary between individuals. This variability is poorly understood and the possibility that psychological factors may play a part has received increasing attention. The documentation of an extensive network of neural and molecular communications between the nervous system and the immune system has fuelled further investigation into precise

mechanisms of interrelated functioning and possible implications of this in relation to pathophysiology. The search continues to elucidate the neuroanatomical and molecular steps between exposure to stress and its effect on immune response, disease susceptibility and outcome.

There is a wealth of literature examining the relationship between stress and disease, but much of this work concerns ailments other than infectious disease such as cancer and cardiac disease [144-147]. The relationship between stressful life events and infectious disease, in particular meningococcal disease, is less extensive.

A true psychoimmunological approach to the study of stress and infectious disease began in earnest in the 1960's with the elucidation of close links between the nervous and immune systems. The idea that stressors of a psychological (ie. no physical contact) nature could cause central nervous system responses that could in turn modulate the immune system led to the concept of neuroimmunomodulation or "psychoneuroimmunology" [143]. This can apply to disease states that are closely regulated by the immune system such as cancer, autoimmune disease and allergy, but may also be relevant when considering infectious disease.

Extensive reviews of the literature have been published by Biondi [143], Cohen [147] and Jemmott [148] amongst others. The bulk of work to date has been performed on animal subjects but there are a number of published works relating to humans. What follows is a summary of the relevant evidence from studies that have been designed to examine the relationship between stress and infectious disease with a review of current theories surrounding psychoneuroimmunological processes.

5.1.2 Psychological Stress; Definition and concepts

Selye introduced the concept of stress in the early 20th century. He defined it as "the sum of all non-specific changes caused by different noxious agents, the rate of wear and tear in the body that accompanies any vital activity and, in a sense, parallel the intensity of life"[149]. Initially the term was applied to stressors of a physical nature, but as the years passed the notion of psychological stress evolved.

Conceptualising the body as an homeostatic organism, others see stress as a state of threatened homeostasis during which the body activates adaptive mechanisms to

maintain equilibrium [¹⁵⁰]. The ability to readjust back to steady state is conceptualised as coping, and excessive changes are proposed to tax the body's capacity for readjustment and thereby cause illness [¹⁵¹]. The ability to readjust is not uniform amongst individuals. Recent attention has highlighted this variability in the response to differing stressors, and research has been directed towards the identification of modifying factors that alter vulnerability to the effects of stress [¹⁵²]. Biological and genetic vulnerabilities to stress, social factors such as social support, and many psychological traits have all been postulated as such modifying factors.

The term "stress" has also been used to denote a stimulus, a response or on occasions the interaction of the two [¹⁴⁸]. However it is now more generally accepted that the term "stressor" be applied to any stimulus that is able to elicit a stress response.

Recent research focuses on psychological stress over and above physical stress. In humans psychological stressors include events such as marriage, death of a loved one or moving house. These are common events that people experience to varying degrees during the course of a lifetime. The cumulative effect of these on health is postulated to be an adverse one [¹⁴⁸]. It is believed that stressors may influence the pathogenesis of disease by causing negative affective states (the "stressed" state) which then influence biological processes or behavioural patterns that in turn increase or decrease disease risk [¹⁴⁷]. This hypothesis forms the basis of the work contained within this chapter.

5.1.3 Psychological Stress in adolescence

Most research to date examining life stress and ill-health has been conducted on an adult population. Much less is known about life stresses acting within the adolescent population [¹⁵³]. During puberty and adolescence major physical, psychological and social changes take place. Therefore, it is not surprising that it is considered an inherently stressful period of development [¹⁵⁴], although fortunately the majority of teenagers do not experience significant psychopathology or persistent distress [¹⁵⁰]. Agnew proposes a model to explore the issue of stress during adolescence [^{155;156}]. He suggests that during early adolescence individuals take on greater responsibility and therefore more frequently find themselves in situations that may be perceived as stressful. In addition, due to their particular stage of cognitive development

adolescents are more likely than adults to see their environment as antagonistic. Perceived stresses therefore assume a larger role in their lives. Some research suggests that adolescents actually experience more stressful events [¹⁵⁷] leading to a combination of greater actual and greater perceived stress. Thirdly Agnew believes that adolescents lack the maturity and power to deal effectively with stressful events. They are consequently more likely to react to stressful events with negative emotions. Added to this are the physical effects of puberty-related hormonal and growth changes [¹⁵⁴]. This combination of factors may result in adolescents being more vulnerable to the effects of stressful life events.

As discussed later in this chapter, stress may predispose to infectious disease through a direct “biological” pathway (see section 5.1.6.1). In addition, stressful life events in adolescence increase the likelihood of deviant behaviours such as illicit drug use, smoking and excessive alcohol consumption [^{158;159}]. This “behavioural pathway” might be an important mechanism by which stress may increase the risk of meningococcal disease (see section 5.1.6.2).

5.1.4 Psychological stress and infectious disease; The evidence for a relationship

In 1953, when talking about patients with pulmonary tuberculosis, Wittkower stated that “ today it is conceded that from eighty to ninety percent of the influences which determine the prognosis are psychological and have to do with the emotional, mental and nervous reactions of the patients.” [¹⁴³]. Pioneering work performed by Ishigami in 1919 demonstrated a link between active disease and stressed patients and postulated an immunological explanation by showing decreases in phagocytic ability of white blood cells during “emotional excitement”. He concluded that the “stress of contemporary life” could impair immunological functioning and decrease resistance to TB [¹⁴⁸]. A number of studies of tuberculosis sufferers since have been able to show a similar relationship. A group of studies on sanatorium workers demonstrated a higher rate of tuberculosis amongst those experiencing more stressful life events [^{160;161}]. Patients admitted to a sanatorium over a 14-year period experienced significantly more stressful life events in the two years preceding admission than in any other year [¹⁶⁰]. In addition mortality from tuberculosis has been shown to be higher amongst divorcees [¹⁶²]. The role of confounding variables such as

homelessness and alcoholism amongst this particular group of patients, as well as the way in which stress was measured, have led to criticisms of methodology but in general a relationship between stressful life events and pulmonary tuberculosis was demonstrable. However, the majority of these studies took place in the 1960's, employed a retrospective design, and were neither randomised nor blinded.

Further epidemiological evidence concerning the association between stressful life events and morbidity or mortality exists but as the majority of this literature does not separate infectious disease from other disease states it is not reviewed here. However, several epidemiological studies have concentrated on infectious causes of morbidity or mortality. For example, separated or divorced adults have six times the likelihood of dying from pneumonia than do married adults [¹⁶³]. Craig and Lin found that elderly psychiatric patients suffered fifty times more deaths from pneumonia when initially hospitalised than age-matched population controls. The effect could not be explained by hospitalisation alone [¹⁶⁴], but in both these studies other confounders are likely to be operating.

5.1.4.1 Viral Upper Respiratory Tract infection

In order to attempt to overcome some of the design problems inherent in many of these early studies, research turned to the study of the common cold, which can be caused by one of more than 100 different viruses [¹⁴⁷].

In work by Boyce et al young children living in families experiencing more life events in the preceding year had viral upper respiratory tract infections of significantly longer duration than children from families experiencing fewer life events. In addition severity of illness was greater in children from families with many life events plus a highly routinized lifestyle. No effect was noted however for frequency of illness although the sample size was small [¹⁶⁵]. It is likely that there are many confounders operating here. In a larger sample of 107 adults and children, risk of upper respiratory tract infection was again positively associated with life event stress [¹⁶⁶].

The acute course and benign outcome of the common cold allows longitudinal studies of subjects undergoing experimental inoculation. This eliminates the

differences between exposure to the source of infection and allows greater precision in study design.

One such study was a large investigation of the influence of psychosocial factors on the common cold performed by Cohen et al [¹⁶⁷]. Four hundred and twenty healthy subjects were inoculated with one of five different common respiratory viruses after psychometric evaluation concerning negative life events for the previous year. Chronic stress was related in a dose-dependant manner to risk of developing disease - in particular, to the phase of active replication of virus within the nasal mucosa rather than clinical symptom expression. The authors were unable to explain this relationship in terms of measured immunological parameters. The development of disease was unrelated to viral type or to prior exposure to the virus. This suggested that the effect of stress acted on a non-immune defence mechanism or on a non-specific immune mechanism. It has been suggested that the observed effect may be due to the effect of stress on nasal mucosal barrier integrity [¹⁶⁸], an interesting concept in relation to what we know about meningococcal disease and nasal mucosal defence (see sections 1.2 and 3.2.2.2). Stress has been associated with oedema, hypersecretion and hyperaemia of the nasal mucosa [¹⁶⁹] and as such could explain the link with invasive viral infection.

Other studies have addressed this relationship and in general confirmed these findings. College students exposed to virus-containing nasal spray and who developed disease were more likely to record recent distress than were those who did not develop disease [¹⁴⁸]. Broadbent et al [¹⁷⁰] found a greater level of clinical symptom expression in experimentally inoculated subjects who scored highly on stress scales, although frequency of recorded infection did not differ. This could be explained by symptom contamination with the scales used to measure stress, or alternatively by psychological factors influencing the perception and reporting of symptoms in stressed individuals. This “illness behaviour” is the phenomenon whereby highly stressed individuals report episodes of upper respiratory tract infection more frequently than non-stressed subjects but have no difference in verified infection rates [^{171;172}].

5.1.4.2 Influenza infection

As discussed earlier in this review, influenza infection may predispose to the development of meningococcal disease (see section 3.2.1.1). Evidence of a link between influenza and life stress is less clear than for the common cold. Development of specific antibody to flu vaccine was unrelated to psychosocial variables, as was development of clinical disease following inoculation [^{173;174}]. Psychologically vulnerable individuals were more likely to report symptoms than non-vulnerable subjects, but there was no difference in actual infection rates [¹⁷¹]. Broadbent found no relationship between infection and any of the measured psychological parameters in subjects experimentally inoculated with influenza [¹⁷⁰]. A study looking prospectively at the development of naturally acquired disease in two groups previously evaluated for individual stress and family relationships found the incidence of disease (clinically, microbiologically and serologically) was greater in stressed, rigid, chaotic families. However individual stress measures were not correlated with disease incidence [¹⁷⁵].

In summary, the evidence from studies is suggestive of a link between life stress and upper respiratory tract disease. It is by no means definitive as the role of illness behaviour in stressed individuals may complicate the picture. The relationship between influenza and psychological stress is even less clear.

5.1.4.3 Latent Virus infections

Of relevance to this thesis is the association between latent viral infection, reactivation and stressful life events, in particular the role of EBV. The possible role of EBV in the pathogenesis of meningococcal disease within the adolescent population has been discussed (see section 3.2.1.3).

Stress or mood alteration and reactivation of latent genital or oral herpes simplex has been extensively studied. Psychological stress may suppress cell-mediated immunity thereby allowing reactivation of latent viruses [¹⁴³]. This would be reflected in higher antibody titres to such latent viruses. A number of studies reviewed by Biondi have demonstrated this effect [¹⁷⁶⁻¹⁸¹]. Sufferers themselves cited emotional upset as a common cause for recurrence of disease [¹⁴⁸] but obviously more than anecdotal evidence is required. Kemeny and colleagues demonstrated decreases in CD8+

lymphocytes during negative mood states, linking this to recurrence of genital herpes [182]. Other studies have shown discordant results. Biondi remains ambivalent about the specific role of stress in the reactivation of herpes simplex infection [143].

EBV acutely infects the host and then remains in a latent form at central nervous system and B lymphocyte level. There are therefore two phases at which perceived stress may be important, that of increasing susceptibility to acute infection and that of reactivation of latent virus. Both retrospective and prospective studies have been applied to the study of stress and acute EBV infection and results of some of these suggest a relationship. University students who were diagnosed as suffering from glandular fever (the clinical syndrome associated with EBV infection) were matched with uninfected controls. Male students with disease reported significantly more life stress than controls, but this result could not be reproduced for female subjects [183]. A large prospective study, included in the review by Jemmott, involved 1,400 military cadets. This study implicated psychosocial stress in both the aetiology and duration of infectious mononucleosis. The author found that those cadets who were highly motivated to achieve but whose performance was deemed to be poor had a greater incidence of clinical disease and a greater length of hospitalisation than those with good performance [148].

A number of studies investigated the association between psychosocial factors and reactivation of latent virus. Glaser et al [184] examined university students who were latent EBV carriers. During exam periods there was a partial reactivation of virus associated with an increase in titres of antibody to antigens expressed early in the course of disease. Together with other work it was thought that this might be as a result of suppression of EBV-specific cytotoxic T-cells. Subjects demonstrated higher titres during the examination period with levels dropping during baseline periods. This suggests poorer immune system control of viral latency [185]. Antibody titres to two other latent herpes viruses, Herpes Simplex Virus Type 1 and Cytomegalovirus, showed the same pattern [185]. Interestingly however, subjects did not experience clinical manifestations of reactivation. This finding has been replicated by Kasl et al who observed that stressed army cadets had an increased risk for seroconversion, higher antibody titres to EBV and longer hospitalisation following seroconversion [186]. Elevated antibody levels to EBV have been found

amongst recently separated women [¹⁷⁸] and amongst caregivers of Alzheimer's patients [¹⁷⁹].

It seems plausible that psychological stress may foster the development of primary infection or viral reactivation. The evidence however is not conclusive and many studies suffer from methodological flaws. There are few studies that have simultaneously demonstrated a trio of high levels of stress alongside compromised immune function and adverse health outcomes.

5.1.5 Psychological Stress and meningococcal disease

Although a body of literature exists on the association between viral infectious diseases and psychosocial stress, much less has been published on meningococcal disease and stressful life events. Apart from tuberculosis, bacterial infections are poorly studied in this context. Bacterial pneumonia has been associated with loss in young widows and widowers [¹⁸⁷] and psychological stress was found to predispose to streptococcal pharyngitis [¹⁸⁸], dental caries and "trenchmouth" [¹⁸⁹] although no measurements of any change in personal health practices were included in these studies. Psychologically vulnerable subjects succumbed to experimental inoculation with tularemia and developed more severe disease than subjects deemed non-vulnerable [¹⁹⁰].

To my knowledge two studies have addressed the relationship between stress and meningococcal disease. The first was a study from Oslo involving 115 patients and 293 population controls [⁴⁴]. In this study stressful life events were defined as participation in athletic competitions, heavy physical work, diving, exposure to hot or cold ambient temperatures and dramatic episodes including grief reactions. Results indicated that experiencing such events prior to illness was significantly more common in cases than in controls, but the case fatality was significantly reduced in the stressed patients compared with the non-stressed patients. Although the results are interesting it is unlikely that the effects seen are a result of psychosocial stress as it has been defined in this study.

A second UK study examined 74 patients [⁴³]. Using a case-control design the events and exposures preceding the onset of confirmed meningococcal infection were studied. A more conventional approach to the measurement of psychosocial stress

was adopted using the Holmes and Rahe Life Events scale [¹⁴⁴]. No relationship was found between meningococcal disease and total life changes score over the preceding six months. However, certain life events such as marital arguments and other marital problems, legal problems and recent changes in living conditions, were significantly more common in the case population than the controls. A holiday in the previous six months was protective.

5.1.6 Psychological Stress and infectious disease; A proposed mechanism

Over the last few decades, extensive work examining the relationship between stressful life events and disease has been published. Much of this evidence suggests that stress may assume the role of a risk factor for disease development. Increasing knowledge of the vast network of connections between the nervous and immune systems at both an anatomical and molecular level [¹⁴²] has fuelled further scientific research into possible mechanisms behind this observation. However it is important to remember that most individuals who experience major life events do not become ill, or they simply experience illnesses comparable to those individuals who have not experienced a major life event. The conceptual framework for the hypothesis that stress can directly lead to disease therefore suggests the triad of demonstrable stress, impaired immune function and actual ill-health [¹⁸⁵]. In order to assign a causal role to stress in the pathogenesis of infectious disease a plausible pathophysiological mechanism needs to be presented.

Biondi proposes two different pathways; biological or direct and behavioural or indirect [¹⁴³]. The influence of stress may be through modulation of the immunocompetence of the host, the anatomical or functional barriers of the host or of the virulence of the infecting organism (biological or direct pathway). It may also be through alteration of the behaviour of the host so as to increase exposure or vulnerability to the pathogen (behavioural or indirect pathway). Most authors agree that it is modulation of the hosts immunocompetence that is the primary mechanism involved although the possible role of nasal mucosal alteration in common cold susceptibility amongst stressed subjects has already been mentioned.

5.1.6.1 The biological pathway

Activation of the central nervous system (CNS) by stressors is the central kingpin in this process. Such activation results in alteration of host defences or organism virulence along either the direct or indirect pathways. The principal operators of the direct or biological pathway are the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Mediators of neuroimmunomodulation are released following activation of these systems by stressors. Corticosteroids (from the hypothalamic-pituitary-adrenal axis) and catecholamines (from the sympathetic nervous system) are well-studied modulators of immune responses [^{143;148}]. Stress also elicits increases in other hormones, namely endogenous opiates, endorphins, growth hormone, prolactin, thyroxine and others [¹⁴⁸]. Many of these may affect immunological processes.

5.1.6.1.1 Hypothalamic-Pituitary Axis

A considerable body of evidence demonstrates high levels of the metabolites of adrenocorticotropin hormone (ACTH) during times of acute stress [^{148;191}]. Glucocorticoids released during stress alter the number, function and distribution of cells involved in the immune response [¹⁴³]. At levels comparable to those produced during stress, corticosteroids are immunosuppressive, leading primarily to a decreased lymphocyte response to mitosis and a decreased killing ability. They may also alter the virulence of infecting organisms [^{143;148}]. In vitro, glucocorticoids can directly enhance viral replication or reactivation [¹⁴³]. In a number of studies, cortisol has been shown to induce replication of latent EBV in vitro [¹⁴³]. However in vivo studies of glucocorticoids and reactivation of latent EBV have not confirmed this hypothesis [¹⁸⁴].

5.1.6.1.2 Sympathetic Nervous System

Although most research has concentrated on the effect of corticosteroids on immune function, evidence is also accumulating on the role of catecholamines. Activation of the sympathetic nervous system leads to an increase in plasma catecholamines. Blood levels significantly increase during periods of experimentally induced stress and fall again following relaxing or non-stimulating cinema films [¹⁴⁸].

Immunomodulation can occur as a direct result of high levels of these substances in vitro, although in vivo experiments are less conclusive. Lymphocytes contain receptors for catecholamines on their surfaces, stimulation of which decreases the cellular response of T cells, B cells and macrophages [¹⁴⁸]. This effect is mediated by cyclic AMP. Production of antibody and interferon is affected and many other functions of the cell appear to be depressed [^{148;185}]. Work by Kiecolt-Glaser et al on 40 medical students during examination periods demonstrated changes in the production of interferon gamma and in both plasma and intracellular cyclic AMP levels from baseline during periods of stress [¹⁸⁵].

5.1.6.2 The behavioural pathway

An important element of the relationship between stress and infection is the indirect or behavioural pathway. Behavioural correlates of the stress reaction are potentially able to influence antimicrobial host defence, exposure to infecting organisms and virulence of such organisms [¹⁴³].

Stressed subjects can be observed to undergo changes in health practices, social relationships and sexual habits that can have an indirect action on host-parasite relationship [^{143;147}]. Behaviours such as alcohol consumption, cigarette smoking and illegal drug-taking may all increase in response to stressful situations in certain individuals. An individual may sleep less, eat erratically or change previous exercise patterns. These elements can all exert their effect on susceptibility to infectious disease. However Cobb et al could not relate verified episodes of upper respiratory tract infection to changes in personal health practices [¹⁶⁶].

In summary, although immune modulation can be demonstrated in response to stress, evidence concerning the biological and clinical significance of this is less clear. Psychological stress can be considered a potential, but by no means a certain, causal cofactor of infectious disease.

5.2 Modifiers of the stress-disease relationship; Social support networks

5.2.1 The role of social support networks in buffering the effects of stress on the immune system

Following the publication of literature on the relationship between stress and ill health, the possible effect of moderating variables is now under investigation. The list of candidate variables, termed “resistance resources” by Antonovsky [¹⁹²], is ever growing and includes concepts such as personality hardiness, exercise and social support. The topic of this discussion is that of social support as a moderator of the adverse effects of stress on health.

A prerequisite for the discussion is the careful definition of social support. Social support as a term has been beset with definitional controversy, which in turn paves the way for measurement controversy. Cobb defines social support as being information available to an individual that leads that individual to believe that he or she is cared for and loved, esteemed and valued, and that he or she belongs to a network of communication and mutual obligation [¹⁹³]. Cobb dichotomises the wider concept of social support based on the above definitions, producing the terms *emotional support* and *esteem support*. Fiore et al reviewed the literature concerning definition and identified five overlapping components of the term social support [¹⁹⁴]. Firstly she identified *cognitive guidance* which conceptualises the need for information and advice from the available social network, and secondly *emotional support* which involves stability, comfort, the need for strong relationships and to be loved and cared for. The third aspect is that of *socialising*, a sense of social integration, belonging to a network of people with shared interests. Fourthly is *tangible assistance* which is the need to be able to rely on concrete or physical assistance when needed. Lastly is the *availability of someone to self-disclose to or to confide in* which needs no further clarification.

There are a number of hypotheses that surround the relationship between life stress and social support. Simplistically it could be speculated that social support networks aid good health as a main effect but this seems not to be the case [¹⁹³⁻¹⁹⁵]. Could social support networks facilitate coping with crisis and change and as such reduce

the impact of such events on health? Many authors support this hypothesis [^{193;195}]. A slightly different perspective adopted by Fiore et al is that it is not necessarily the availability of the support that is important in producing effects per se, but lack of support that functions as an additional stressor. In other words, the positive effects of social support availability are not as important as the negative effects of its perceived unavailability [¹⁹⁴]. In this model a perceived lack of support functions as an additional stressor. This means that when asking an individual about perceived adequacy of support one is receiving a summary assessment composed of the negative as well as the positive aspects of their social network. Fiore et al argues that this produces an artificial separation of support and stress in the literature [¹⁹⁴].

Another important element of the social support concept is that of quantity versus quality. Although quantifying social support is an easier task (frequency of contacts, number of network members etc) it does not account for effective coping or adequacy of the support available. Only weak associations have been demonstrated for the amount of support received, whilst the perceived quality of support has repeatedly been found to relate to both mental and physical health [¹⁹⁴].

At this point it seems prudent to further evaluate some of the published literature on the subject. Cobb approaches his review of published literature by studying different phases of the human life cycle [¹⁹³]. He reviews data from Nuckolls et al on childbirth [¹⁹⁶], arguing that in a group of women assessed prior to delivery a higher rate of complications was found in the high stress/low support group than in the other groups. These data were dichotomised into high and low stress/ support groups based on a split at the median and no mention of possible confounders was made. In a study of women requesting abortion and being denied [¹⁹⁷], low birth weight in this group as compared to a control group was postulated by Cobb to be directly due to the fact that the most common reason for rejection of a pregnancy by women is inadequate social support. This assumption is questionable. He goes on to discuss data pertaining to the development of the infant and young child in relation to social support, but does not produce strong enough arguments for comment here.

Evidence surrounding measured ill health provides more suggestive data. Holmes et al found that outcome of tuberculosis treatment could be predicted by placing the patient on a social support scale. All treatment failures were in the lowest third of

scores [¹⁹⁸]. Cobb comments that TB is a disease of social isolation and low social support, but again fails to mention that TB is also a disease of immigrants, alcoholics and the destitute where drug compliance is a major issue. Clearly an association is likely to exist, but causation is questionable. More convincing is a study performed in Seattle on adult asthmatics and the need for corticosteroid therapy [¹⁹⁹]. Again those with low stress scores needed smaller doses of corticosteroids. Of patients placed in the high stress scores groups, those with low social support needed four times the dose of corticosteroids than did those in the high social support group. No data controlling for severity was presented.

In a prospective study of 107 adults, designed to look at life events, coping style, social support and the development of respiratory illness over a 15-week period, Cobb et al found unexpected results [¹⁶⁶]. Social support was measured using the Social Support Questionnaire developed by Sarason et al [²⁰⁰]. Cobb found that the social support score as a whole was not related to the risk of developing infectious illness, but that under conditions of low life stress, high social support was associated with a reduced incidence of infection. One suggested interpretation of this result was that the protective effects of support were submerged under high stress conditions, or an alternative possibility is that under stressed conditions people with large supportive networks increase their exposure to infectious agents. Social support was not found to be as important a moderator as coping style.

Kobasa et al [¹⁹⁵] examined three “resistance resources” in relation to the development of illness. Resistance resources are variables that moderate the relationship between stressful events and illness symptoms. This study considered three resources, personal hardiness, exercise and social support, although the list is ever-expanding. Subjects were 85 stressed businessmen in Chicago. A direct correlation was found between the number of resistance resources and fewer symptoms. Of the three resources measured, personal hardiness was the most important buffer, with the effect of social support being relatively small.

The effect of other stressful life events can be examined in relation to available support. Termination of employment can be a highly stress-evoking event for those supporting a family. Gore et al studied 100 men who had been sacked from employment and 74 controls [²⁰¹] and found that men with low social support had

higher cholesterol and uric acid levels in the serum, but complained less of symptoms. There was no relationship with hypertension or peptic ulcer disease.

Fiore and colleagues studied the spouses of patients with Alzheimer's disease [¹⁹⁴] to address the hypothesis that it is not necessarily the positive effect of support that exerts a main effect, but the lack of expected support that acts as a separate stressor, increasing the likelihood of disease. All five elements of social support previously stated were tested (socialising, tangible assistance, cognitive guidance, emotional support, self-disclosure). Forty-four spouses of patients with Alzheimer's disease were evaluated for depression using a standardised depression inventory. Perceived upset at absence of support was analysed in relation to depression, as was the perceived helpfulness of support. In all areas of support the correlation between perceived upset and depression was highly significant, while in no case did perceived helpfulness relate significantly to depression. In addition the upset ratings alone predicted depression better than the helpfulness to upset ratios. This study produced findings consistent with the notion that it is the extent of disappointment with the social network that was the best predictor of depression in a chronically stressed population. The perceived supportiveness of the network did not predict depression.

From the results of the studies reviewed, it is clear firstly, that findings reflect the complexity of human relationships and the responses to stress and support. In addition, the conclusions drawn from some of these studies may reflect the social and political context of the time in which they were performed. It would be fair to conclude that there appears to be a positive relationship between stress and networks of perceived social support no matter how small. However the ability of this social network to produce actual changes in clinical symptomatology and disease is unclear. Fiore et al postulate a minimal level of support above which individuals receive no additional buffering benefit although she is hesitant to suggest the complete absence of any buffering effect at all. She emphasises that lack of support acting as a stressor is more relevant.

Intuitively, supportive interactions between people must be both important and beneficial. What is more difficult is the assembling of the evidence.

5.2.2 Social support networks in adolescence

There is a relative paucity of data for adolescents on social support networks. This is a time during which adolescents' support from the peer group increases while support from family decreases [²⁰²]. If family support remains high, the effect of life events on a single variable relating to adjustment, namely drug use, appears to be modified [²⁰³]. Life events also appear to have a stronger impact on drug use in females when family support is low [²⁰²]. However, during adolescence it has been argued that family relationships become a source of stress rather than support as peer group demands and pressures produce conflict within the family [²⁰⁴]. Optimal families respect and encourage the adolescent's independence while providing a secure base in times of need [²⁰⁴]. However, this is clearly a difficult task and for many adolescents the family climate is less than ideal. They may therefore be faced with a situation of loss of one social support network (namely the family) coupled with increasing stress resulting from family conflict.

The issue of peer group support versus family support has not been widely investigated within the context of stress and disease in the adolescent population. Shulman examined adolescent coping in relation to family and peer group support. Not surprisingly, support from both peers and family was found to facilitate coping and buffer stress in young, especially female, adolescents [²⁰⁴]. However, Rowilson found no support for the hypothesis that general social support networks had any buffering effect on major life events within an adolescent population [²⁰⁵].

5.2.3 Other moderating variables

Since the elucidation of a relationship between psychological stress and disease, there has been an ongoing search for factors that might mediate the impact of stress and help to explain inter-individual differences in response to stress. As discussed, such moderating variables might be psychological (eg. hardiness, coping style), social (eg. social support, social competence) or physical (eg. race, gender). Social support has generally been the factor most widely studied and accepted as having an effect. The part that exercise may have to play in predisposing to or protecting against meningococcal disease has been discussed (see section 4.5). The possible role of exercise as a buffer of the effect of life stress has received increasing attention. It seems accepted that the negative impact of stress on physical health declines, at least

up to a point, as the amount of exercise increases [¹⁹⁵] and this relationship has been replicated within an adolescent population [^{206;207}]

5.3 Summary

For more than 100 years a relationship between stress and infectious disease has been recognised. More recently there has been an ever-increasing understanding of how such a relationship might be mediated. In vitro assays have demonstrated a deleterious effect of stress on immunological processes, such that a similar in vivo effect is a real possibility. Behaviour modification in response to stress is also well demonstrated as a mediating pathway.

Social support networks may act as a buffer to the effects of highly stressful situations. In addition their absence might act as an individual stressor.

Adolescents experience specific stressors that can be measured using validated scales (see section 6.2.3.1.8). It is a life-phase that is associated with the development of close personal relationships and peer grouping, and with the development of an expanding network of social supports. It is also characterised by increasing conflict within the family unit.

Adolescents experience a higher incidence of invasive meningococcal infection than might be anticipated. The role of stress and social support in the aetiology of meningococcal disease within the teenage population has not been investigated.

5.4 Aims of this study

Adolescence is a period of dramatic psychological adjustment. It marks the transition from childhood to adulthood. Specific sources of stress are common during this phase of life [^{154;207}]. Meningococcal disease displays a biphasic incidence pattern with a relative peak in incidence during the teenage years which remains largely unexplained. A relationship between psychological stress and infectious disease has been demonstrated and extensively reviewed [^{143;147;148;185}]. The possible mediating effect of social support networks on this relationship has been explained (see section 5.2.1). However the role of specific stressors and networks of social supports during adolescence as a risk factor for the development of meningococcal disease have not been investigated. This study employs validated

scales for measuring both life stress and social support networks in the teenage population and aims to investigate a possible link between these variables and invasive meningococcal infection.

6 Methods

6.1 Study design

I conducted a prospective case-control study covering six regions of England. The aim was to investigate the risk factors for the development of MD in adolescents. Study subjects were young people aged 15 to 19 years inclusive admitted to hospital with a diagnosis of suspected MD between January 4th 1999 and June 9th 2000. Each case had a single control. Controls were age- and sex-matched and identified from the GP list of the index case.

MD is a rare disease making a case-control design appropriate for studying risk factors. In determining sample size, 90% power to detect odds ratios of between 2 and 5.4 at the 95% confidence level was assumed. The sample size was based on effect size rather than precision. Five main variables were considered for sample size calculations (see table 1). These examples were based on known or estimated rates of factors occurring in the general population. Although power calculations were only performed on these five variables, other exposures were measured to deal with possible confounding variables and to generate hypotheses for future studies.

Table 1. Sample size calculations

Risk factor	Expected group prevalence	Number needed in each group
Smoking	cases 30%	116
	controls 12%	116
Mouth kissing (> 1 contact in previous fortnight)	cases 40%	118
	controls 20%	118
MBL (Codon 54 homozygosity rate)	cases 12.5%	163
	controls 2.5%	163
D. Life event scale (score >4)	cases 30%	116
	controls 12%	116
E. Elite athletics (>14 hrs training/week)	cases 15%	134
	controls 3%	134

6.2 Study protocol

6.2.1 Multi-centre design

In order to enroll the numbers required, cases were recruited from six of the eight regions of England. The regions selected were North Thames, South Thames, South and West, Anglia and Oxford, West Midlands and Trent. Cases were also included from a single health authority in Yorkshire. These regions were chosen on the basis of the projected number of cases of meningococcal disease occurring in this age group nationally, together with the logistic considerations of travelling from London to visit study subjects. Thus the six regions in closest proximity to London were selected. Sufficient numbers of cases of MD in the age group under study were likely to occur in these regions over the period of study. As these regions contained a large proportion of the cases occurring nationally, cases were assumed to be representative of the population at large.

The decision to include a single Health Authority in Yorkshire was made because of the enthusiasm of the local collaborator to participate.

6.2.2 Issues of collaboration

In order to minimise selection bias, it was important that a high percentage of the total number of cases occurring in the six regions under study were notified to the study centre for possible inclusion. In order to achieve this, extensive collaboration with health professionals was required.

Referral of cases to the study centre came from four sources; consultants in communicable disease control (CCDC's), the Manchester Reference Laboratory, hospital clinicians and the Meningitis Research Foundation.

6.2.2.1 Consultants in Communicable Disease Control

As MD is a notifiable disease, doctors have a statutory requirement to inform the local public health department if they suspect a case under their care. Consultants in Communicable Disease Control are responsible for co-ordinating the response to notifications of MD and are therefore made aware of possible cases early in the

course of disease. As they have an interest in public health issues surrounding MD, CCDC's were the primary source of referrals to the study.

However, CCDC's have large workloads and requests for information or invitations to participate in research studies are frequent occurrences in their everyday working life. In order to maximise co-operation, it was important that CCDC's were fully informed about the possible public health benefits of this research. A database of CCDC's in the six regions under study was obtained from the Centre for Disease Surveillance and Control in Colindale, North London. All CCDC's were sent an invitation to participate in the research (see appendix 1) together with a synopsis of the protocol and a small poster for an office wall or desk to act as an aide-memoir. A copy of the full study protocol was sent if requested. A single telephone notification to the study centre or mobile telephone number (out of office hours) was requested as soon as possible after the CCDC had been notified of a possible case.

Later in the course of the study a mousemat was designed and sent to all CCDC's to further maximise recruitment.

6.2.2.2 The Manchester Reference Laboratory

The Manchester Reference Laboratory processes a high proportion of samples collected for the diagnosis of meningococcal infection nationally and is a centre of expertise in the laboratory diagnosis of this disease. Contact was established early regarding case recruitment and sample collection. The laboratory holds a database of all the samples they receive and process. Daily communication was established between the study centre and Manchester and a list of cases sent by e-mail to the study centre, detailing all the young people between 15 and 19 years for whom a sample had been received for the diagnosis of possible meningococcal infection. Subsequent liaison with the admitting hospital revealed their suitability for inclusion in the study.

6.2.2.3 Hospital clinicians

It became clear that notification rates were unlikely to be sufficiently complete if these two methods alone were relied upon. Approaches to further maximise recruitment were therefore devised.

Directors of Intensive Care Units and consultant paediatricians and physicians within the six regions were invited to refer patients to the study centre. They were contacted by letter explaining the study (see appendices 2 and 3) and were sent a small aide-memoir poster for display on wards or Intensive Care Units.

6.2.2.4 Meningitis Research Foundation

The Meningitis Research Foundation is a national registered charity established in 1989. As well as providing information and support to the public relating to meningitis and septicaemia, it also funds research into the disease. As the funding body for this study, MRF was able to provide information to members of the public who contacted their helpline relating to the study. Some of these people agreed to have their contact details passed on to the study centre for possible inclusion.

Frequent feedback to referring sources was essential to maintain co-operative links and encourage recruitment. A quarterly communication was written and sent to all notifying centres during the period of data collection.

6.2.3 Development of the questionnaire

The questionnaire was developed with reference to that used in the West Country Meningitis Study [⁴³]. The West Country Meningitis Study was a case-control study designed to examine risk factors for the development of MD over a wide age range. To ensure comparability and repeatability the questionnaire employed in that study was used as a basis for the development of the questionnaire used in this adolescent population. However, it was necessary to adapt the questionnaire in order to address the specific hypotheses of this study. The literature concerning adolescent behaviour and measurement techniques was referenced where appropriate and as many previously validated scales as possible included. Standard methods of measurement of common variables (eg. social class) were used. Questions related to the two week period prior to admission to hospital (cases) or the two week period immediately preceding interview (controls). A two-week period was chosen for comparability between other major risk factor studies that have also selected this time frame [^{43;119}]. Although other studies have employed a one month period preceding illness or interview [^{41;84}] recall bias becomes more problematic. There are no studies that use a shorter time frame.

The questionnaire consists of two parts (see appendix 24). Each part contains sections relating to hypotheses under examination. What follows is a brief description of the methods involved in the production of each section.

6.2.3.1 Part 1 - Questionnaire for young people

6.2.3.1.1 Section A - Demography

This section was designed to collect basic demographic information. The classification of ethnic origin was that used by the Office of Population Censuses and Surveys [⁵⁸].

6.2.3.1.2 Section B - Health

This section was designed to examine the relationship between predisposing respiratory viral infection and the development of MD. Data collected here formed a subjective measure of viral infection. The West Country Meningitis Study used any 3 out of 5 possible symptoms to indicate the presence of an influenza-like preceding illness (personal communication Stanwell Smith, CPHL). These symptoms were; sore throat, fever, headache, muscle/joint aches and dry cough. For this questionnaire, two additional possible symptoms of respiratory viral illness were chosen, namely shivering/rigors and running nose and the presence of any three of these seven taken as suggestive of disease. The decision to include the extra symptoms was deliberate as this study examined a wider spectrum of respiratory viral infection than the West Country Study. Cases were asked whether they had suffered any illness in the fortnight period from which they recovered prior to developing meningococcal disease (a likely preceding illness) as well as any illness immediately prior to admission to hospital (a likely prodromal illness). They were asked to choose any symptoms from the same set of seven symptoms that might have been present during either of these illnesses. This was an attempt to separate preceding respiratory viral illness from the prodromal illness often associated with meningococcal disease. Symptom profiles for these two entities would be further analysed in order to control for the confounding effect of prodromal illness during analysis.

This symptom-based definition is a somewhat arbitrary and subjective method of assessing viral infection. We aimed to offset this subjectivity by collecting samples from study subjects for the laboratory diagnosis of respiratory viral infection (see section 6.6). It is possible, however, that predisposing respiratory viral infections may have been cleared by the host by the time samples were collected. Therefore, as the questionnaire asked about symptoms during the two-week period prior to meningococcal infection, this subjective assessment of respiratory viral infection could be important. As there is no widely standardised grouping of symptoms that can reliably be used to indicate the presence of a respiratory viral infection (personal communication Fleming, RCGP), it seemed reasonable to employ those used by Stanwell Smith.

6.2.3.1.3 Sections C&D - Occupation & Accommodation

Tickboxes were used to record subject occupation and place of work. Information about the type of accommodation and space available was collected and a crowding index calculated. The same crowding index as that used in the West Country Study was employed and consisted of the number of people in a household divided by the number of rooms within that household. As in the West Country Study, bathrooms, toilets and hallways were excluded from the calculation.

A household was defined as those people with whom the subject lives and shares meals. This is also the definition used by the Office of Population Censuses and Surveys [62]. If the subject was a student in a communal living arrangement, the definition of household was that used when defining household contacts for the distribution of antibiotic prophylaxis during meningococcal outbreaks (Personal communication, J Stuart). This was defined as those people with whom kitchen and bathroom facilities are shared.

6.2.3.1.4 Section E - Social contacts

This section consisted of a number of questions relating to intimate (deep kissing) or close social contact. Subjects were asked to state the number of kissing contacts for the fortnight period before disease or interview (both intimate and social). Defining a subject's close contacts, other than their kissing contacts, was difficult. Each

subject was asked to list the people they felt to be their close contacts during the specified fortnight period. The only guidance given was that these should not be work colleagues unless they were seen outside work, and that each must have been seen at least twice during the specified period.

6.2.3.1.5 Section F - Risk taking behaviour; smoking, alcohol and illicit drugs

Major study hypotheses concerned the relationship between social behaviours that develop during the adolescent phase of life and the development of invasive MD.

6.2.3.1.5.1 Cigarette smoke

The questionnaire addressed adolescent cigarette smoke exposure; active smoking, passive smoke exposure and exposure to smokers themselves. These different types of smoke exposure might confer separate risks of invasive disease, acting through different aetiological mechanisms. Although necessary, it was difficult to disentangle these three separate exposures within the confines of a single section of the questionnaire. A small number of questions were directed at ascertaining the active smoking behaviour of the subject. These documented the average number of cigarettes smoked per day and the total number smoked in the specified two-week period. Smokers were classified into light, moderate and heavy smokers according to the number of cigarettes smoked per day to detect any dose-response relationship between meningococcal carriage or invasive disease and cigarette smoke exposure [76].

Passive exposure to cigarette smoke is difficult to quantify. Passive smoking occurs within a household if household members smoke. Therefore the number of household members that smoke inside the household, and the number of cigarettes smoked by each household member were chosen as quantifiable measures. A single question concerning exposure to smoke at work was included.

In order to attempt to measure exposure to smokers themselves separately from exposure to smoke, the number of close contacts who smoked and the number of

cigarettes smoked by household members outside the home, were recorded as variables.

6.2.3.1.5.2 Alcohol

Two elements needed to be assessed. Firstly total alcohol consumption in the specified fortnight period was recorded. Secondly the number of episodes of binge consumption of alcohol in the fortnight period was recorded. This number was the total of the number of hangovers experienced plus the number of times the subject was of the opinion that they had consumed an excessive amount of alcohol but did not experience a hangover.

6.2.3.1.5.3 Illicit drugs

Questions were compiled relating to the number of episodes of illicit drug use in the specified fortnight period and the particular type of drug used. In addition, mode of ingestion of the drug (sniffed or smoked) was recorded because of the possible effect of this on the nasopharyngeal mucosal barrier.

6.2.3.1.6 Section G - Leisure activities

Certain leisure activities, such as attendance at bars or nightclubs (see sections 4.4.1.2.1) or religious observance (see section 4.6) may be associated with either an increased or decreased risk of developing invasive MD. In order to examine the leisure activities of this cohort of teenagers, a simplified version of the grid employed by The West Country Meningitis Study was developed. This looked at places of leisure and the number of times each place was visited in the specified two-week period. The grid was simplified by excluding variables that were considered not directly relevant to the hypotheses under examination in this study (eg. Visits to shopping malls, trips in crowded elevators). The number of times each place was visited in the fortnight period was classified into four groups by frequency.

6.2.3.1.7 Section H - Sporting activities

A simple but objective way of measuring different levels of physical activity was needed for this section of the questionnaire. A comprehensive literature review was

undertaken to investigate existing scales for the measurement of physical activity and to find a suitable method for this study [²⁰⁸⁻²¹⁶]. Many of the methods used for measurement of physical activity are complex and involve computation of calorific expenditure. They were unsuitable for the purposes of this study because they are time-consuming and often involve complex physical measurement. As the possible relationship between physical activity and disease was not of high priority, a simpler method of measurement was used.

A standardised and validated integrated compendium for coding physical activity was identified [²¹⁷] which could be used to calculate a sports score. This could act as a single numerical variable for analysis and had the advantage of being comparable across studies.

6.2.3.1.8 Section J - The measurement of life stress in adolescents

6.2.3.1.8.1 Historical perspective

The objective measurement of stress was first attempted by Holmes and Rahe in 1967 with the Schedule of Recent Experiences (SRE) [¹⁴⁴]. They claimed that an estimation of life stress was best obtained by measuring the number of events over a period of life that were likely to produce stress. They hypothesised that only major events would be important (eg. births, deaths, marriages, job changes) and termed these Life Events. Life Events could be positive (eg. birth of a child) or negative (eg. death of a relative). Holmes and Rahe claimed that an objective measurement of life stress could be obtained by the summation of such events over a defined preceding period.

This concept was based on Selye's theory that all stressors have similar non-specific physiological effects, varying only in the amount of the effect [¹⁴⁹]. This led Holmes and Rahe to use the concept of Life Change Units as their method of weighting the effects that different Life Events may have. They claimed that both positive and negative life events could produce stress and therefore require life change in response. Weights were obtained using a sample of Mid-Western whites, assigning numerical values to events in comparison to an index weight pre-assigned by Holmes and Rahe.

In the twenty-five years since the original SRE, there has been much criticism of this original paradigm, disagreement about exactly what constitutes a Life Event, and the introduction of new weighting and scaling methods.

In 1971 Paykel proposed that it was the undesirability of the event rather than the life change necessary that was the key element of life events' association with illness [218]. Studies since have supported Paykel's assertion, finding that positive life events have no association with ill-health [219-221]. It has also been recognised that positive life events give no protection against stress-related diseases [220;221].

6.2.3.1.8.2 Weighting issues

There are two basic approaches to calculating the life event score once the type of events to be considered have been decided. The number of events within the fixed time period can be summed to produce a stress score. Alternatively, based on the belief that certain events are more psychologically or physiologically stressful than others, events can be weighted according to their degree of significance.

Although weighted scores might appear to produce a more accurate picture of an individual's stress, studies perhaps surprisingly indicate that a simple unweighted count of events over time provides similar results to weighted scores. [219;222].

6.2.3.1.8.3 Methodology

There are two ways of eliciting life event information; the self-report scale as pioneered by Holmes & Rahe, and the semi-structured interview introduced by Brown & Harris in 1978.

Self-report scales are checklists of life events filled in by the respondent, indicating which events have occurred in their life in a defined period of time. Most Life Event studies use some form of self-report in spite of ongoing criticism [223;224]. The advantage of self-report is speed and ease of administration and analysis, hence low cost [225]. However, this method of data collection has drawbacks. The researcher gains no detailed knowledge of the nature, significance or time scale of the event [225]. Secondly, events may be missed as the respondent is given a finite list of possibilities [226]. As it is usually only possible to record one instance of each event,

repeated events are missed. Thirdly, in studies that attempt to associate life events with the onset of disease, there may be "Effect after Meaning" i.e. the re-evaluation of the significance of events by the patient in an attempt to explain or justify their present condition [²²⁵] This is mostly a concern and would be most marked where respondents are aware of the study intent. Lastly accuracy of recall and reliability and the possibility of symptom contamination (see section 6.2.3.1.8.6) are of concern.

In 1983 Paykel suggested that directed interviews were the most valid way of assessing life events [²²³]. Although this method places the events in context and claims higher reliability and less fall-off in reporting of events with time than self-reports [²²³], it does have important disadvantages. Interviews are intensive of staff and patient time with high resultant cost. There is also the possibility of interviewer bias [²²³].

Consequently, in most recent literature self-report scales continue to be used in view of their greater suitability to most research budgets and staffing levels.

6.2.3.1.8.4 Recall periods

Most life event instruments use a 12 month recall period [^{220;227}]. Others suggest that shorter periods such as 6 month are more valid [¹⁵⁷]. A number of issues arise with long reporting periods. Fall-off in reporting is a major concern. Paykel suggested that fall-off rates varied from about 1% per month for interview techniques, to 3-4% per month for self-reports [²²³]. This was felt to be an acceptable loss of information, as distant events that are forgotten were assumed to be unlikely to have had significant distress associated with them.

6.2.3.1.8.5 Reliability and validity

It is important to establish the reliability of psychological instruments. Most estimates of reliability depend on the Test-Retest method.

Assessing the validity of life event measures is conceptually difficult. The common approach has been to compare the respondent's score to that of his/her spouse or parent. This relies on what may be a false assumption, that the co-informant has

extensive knowledge of the events in the respondent's life [²²⁴]. Validity is likely to be underestimated using this method.

6.2.3.1.8.6 Symptom contamination

Symptom contamination of scales occurs when life event items on the scale overlap with symptoms of disease, either physical or psychological. Symptomatic patients may then report increased rates of events. Holmes and Rahe's original SRE is highly contaminated with symptoms, and this has led some authors to claim that all work performed with this instrument in the last two decades is invalid [²²⁸].

Zimmerman [²²⁹] points out that although it is tempting to exclude all events that are not totally independent, this would lead to the loss of valuable data. Studies conducted on the SRE demonstrate that analysis of results before and after the removal of potentially contaminated events, reveal that inclusion of non-independent events does not alter resulting correlations. The conclusion is that although symptom contamination should be guarded against, it is of only minor importance.

6.2.3.1.8.7 The method employed for this study

The Adolescent-Family Inventory of Life Events and Changes or "A-File" [²³⁰] is a 50-item self-report instrument designed to record life events and changes experienced by an adolescent in the year preceding the interview. The scale was developed and validated for completion by subjects' aged 12 to 18 years and gives a total score for family life changes. It is based on the idea that changes within the family may have far-reaching effects on its adolescent members. A single numerical score is produced from the sum of the number of positive responses, no weighting measure is used. A recall period of one year is employed which is a suitable length of time enabling incorporation of important life events whilst not adversely affecting accuracy of recall.

McCubbin et al reported test-retest reliability for his Adolescent- Family Inventory of Life Events of 0.80 over a 4 to 5 week period [²³⁰] which is an acceptable reliability.

The A-File does not include symptoms as possible life events thus avoiding the possibility of symptom contamination in any observed relationship between stressful life events and disease [²²⁹].

An important aspect of the A-File is the inclusion in the scale of positive life events, for example the birth of a sibling or the marriage of a family member. To many, these events might be construed as positive. As discussed above, the consensus from the literature is that it is only negative life event stress that has an adverse effect on health. However, adolescent scales are more concerned with the dynamics of the family system rather than with the individual. The effect of such “positive” events within a family may be very different from the effect of that event on an individual (personal communication R Viner).

In order to add a second dimension to the A-File without necessarily affecting the validity of the basic measure, a linear scale was added to each of the 50 items. Subjects were then asked to rate the distress caused by each life event on this scale. This provides a measure of perceived distress for each item and attempts to overcome any criticisms surrounding the use of a non-weighted measure.

Two scores were therefore produced from the A-File, the total family life changes score and the total distress score.

6.2.3.1.9 Section K - Social support

As discussed in section 5.2.1, the negative effects of stressful life events on health may be buffered by a supportive social network. The lack of adequate social support or unmet expectations of support may act as an additional stressor.

Sarason developed a validated scale for measurement of the number and perceived quality of an individual’s social supports [²⁰⁰]. The scale in full consists of 27 items, but is validated if only three or six of these items are employed [²⁰⁰]. The decision was made to employ six items.

6.2.3.2 Part 2 - Questionnaire for Head of Household

A separate questionnaire was devised for the subject’s Head of Household. This was done in order to classify socio-economic status accurately. The subject group studied

here is one for which classification of socio-economic status can be difficult. Socio-economic status can be assigned to an individual in a number of different ways. Head of Household occupation, car ownership, home ownership and postcode of residence can all be employed as measures [²³¹⁻²³³], but this requires Head of Household for this population to be accurately defined. In this study Head of Household was defined as follows;

If the subject was living at the parental home or was a student studying away from home, the Head of Household was the father, or the mother if the father was absent. This included students living permanently in shared student accommodation. If the subject was independently living they act as their own Head of Household. If the subject was female and married or co-habiting with a male partner, then convention dictates the male acts as Head of Household.

6.2.3.2.1 Section A - Postal address

The postal address of the Head of Household including the postcode was recorded along with that of the General Practitioner. Postcode can be employed as one measure of the socio-economic status of the subject [²³²].

6.2.3.2.2 Section B - Family Health

A short series of questions regarding family history of infectious disease were addressed to the Head of Household. If the subject was acting as his/her own Head of Household and could not answer this set of questions, the parents were contacted. A record was made of relevant vaccination history, especially that of meningococcal C vaccine. Birth weight, gestation and the duration of breast-feeding were also recorded.

6.2.3.2.3 Section C - Head of Household Occupation

Head of Household occupation, car ownership and home ownership were recorded.

6.2.3.2.4 Section D – Hospital information

A final page of the questionnaire was sent to the hospital consultant in charge of the subjects' care. Relevant microbiological information was requested and information collected on whether the subject had been admitted to intensive care or not and, if so, the number of nights spent on the intensive care unit. This provided an approximate measure of disease severity.

6.2.3.3 The questionnaire and recall bias

The case control design of a study is criticised for being subject to bias [²³⁴]. Recall bias is present when a systematic difference occurs in the way that cases and controls recall events. In this study subjects were being asked about events that had occurred in the past. This may lead to significant recall bias.

Case subjects were asked about the two week period leading up to their admission to hospital. Detailed information was sought regarding social behaviours during this period of time. Case subjects would be unlikely to have great difficulty recalling this period of time as it led up to their admission to hospital. However, there were two possible time frame options when considering control subjects. The most logical choice might seem to be exactly the same fortnight period as the index case. However, as control interviews take place at a varying length of time after admission of the case, this would result in control subjects being asked for detailed information about what is for them an arbitrary two-week period that they would probably find difficult to recall. A decision was therefore taken that control subjects would be asked about the two-week period immediately preceding the interview. Although there is a temporal difference between these two fortnight periods, the detrimental effects of this would be less than that of the potential recall bias incurred. Once this decision was reached, it became important to minimise the length of time between the admission of the case to hospital and interview of the control.

6.2.3.4 Validation issues

When a questionnaire is employed to measure a set of variables, it is important that it measures those variables reliably and with repeatability. Questionnaires should contain questions that have been validated wherever possible.

The questionnaire developed for this study included validated scales. The A-File, Sarason's Social Support Scale and the Compendium of Physical Activity have all been employed in other work.

A main study hypothesis was the relationship between respiratory viral infection and MD. Questions were asked about symptoms of respiratory infections during the specified fortnight period. In order to validate these data, biological samples were collected from cases and controls at pre-specified times. These were analysed for the presence of specific respiratory viruses, so providing an objective assessment of predisposing viral infection.

6.3 Ethics approval

The study protocol and supporting documentation was submitted to the North Thames Multi-centre Research Ethics Committee in September 1998. After several amendments final approval was granted 2 months later. The proposal was then submitted to 125 Local Research Ethics Committees covering the hospital trusts from which patients may be recruited.

Local Research Ethics Committees (LREC's) request varying numbers of copies of documentation to support an application. Although their remit is to consider locally pertinent issues only and to communicate a decision to the researcher within three weeks of receipt of the application, this did not universally occur. The result of this was that the study incurred large unforeseen expenses eg. in photocopying and administrative costs, and that study commencement was delayed.

Consequently this process became the subject of a paper published in the British Medical Journal (see appendix 25)

6.4 Subject recruitment

6.4.1 Case Recruitment

Notification to the study centre was via telephone notification from CCDCs', hospital clinicians or the Meningitis Research Foundation Helpline staff. In addition the daily e-mail list from the Manchester Reference Laboratory was used (see section 6.2.2). As a definitive diagnosis of MD is not usually confirmed by laboratory tests

in the early stages of admission to hospital, inclusion in the study depended on MD being the attending clinicians' primary working diagnosis. Laboratory confirmation was sought at a later date. All subjects had to be between fifteen and nineteen years of age on the day of recruitment and admitted to a hospital in one of the six regions of England covered by the study. Local Research Ethics Committee approval had to be in place.

Following referral to the study centre, the attending consultant clinician was contacted to obtain consent to approach the patient and family. If MD was the primary diagnosis, information sheets for the patient and family were faxed through to the admitting hospital (see appendix 9, 10 and 12) and introduced to the patient by the attending clinician. Informed consent was obtained by the attending clinician from all patients (see appendix 13). If the patient was too unwell to provide informed consent, consent for initial involvement in the study was obtained from the next-of-kin (see appendix 15).

6.4.1.1 Ethical considerations in case recruitment

The particular age of the cohort of patients used for this study provided an interesting ethical debate about the issues of consent in teenagers and young people. Since the Gillick ruling, young people under the age of 16 can give informed consent if judged to be competent in understanding the issues involved [²³⁵]. As quoted in the General Medical Council's publication of 1998 "In general, a competent child will be able to understand the nature, purpose and possible consequences of the proposed investigation or treatment, as well as the consequences of non-treatment." [⁶⁰]

The North Thames Multi-centre Research Ethics Committee (MREC) judged all our patients competent to make an informed decision about inclusion in the study, provided the attending clinician or parents had no obvious concerns. Although parents did not have to provide their consent under these circumstances, it was considered good practice both by the North Thames MREC and by the study team to gain parental assent to inclusion, and this was universally done.

The Local Research Ethics Committees (LREC) did not universally accept the decision regarding consent in subjects under 16 years of age. One LREC refused approval for patients in their area who were under the age of 16 to be approached for

entry into the study. In practice most young people gave consent after discussion with one or both parents.

Once consent was obtained blood and swab samples were collected from the subject by the local hospital's admitting team. Addresses and telephone numbers of the subject and GP were requested. Discharge from hospital was confirmed by discussion with the nursing or medical staff prior to any subject being contacted at home for the interview phase of the study. This was done to eliminate the chance of contacting a bereaved family and causing unnecessary distress.

6.4.1.2 Exclusion criteria

Subjects with a poor working knowledge of the English language were excluded as it would not be possible for them to give fully informed consent or to complete the questionnaire. The logistical and financial constraints of identifying a suitable interpreter at a distant hospital at short notice were too great for this to be considered.

Those with an underlying immunodeficiency state or with a neurological shunt in-situ were excluded. This is because their background susceptibility to disease may be higher than in the general population and depend on different factors.

Subjects who died were also excluded. MD can be a rapidly fatal disease. Under these circumstances it would be ethically inappropriate to seek consent from relatives for inclusion in a study. In addition, interview data would not be obtainable from the deceased subject and would therefore have to be sought by proxy. Due to the personal and sensitive nature of the data collected, the bias introduced by seeking this by proxy was judged to be greater than the bias introduced by exclusion of those cases with such severe disease.

6.4.2 Control recruitment

The controls selected in this study were age and sex matched controls from the GP list of the index case. The selection of the control group for a case-control study is of paramount importance. The selected controls must be comparable to the source population of the cases, and represent the population of individuals who would have been selected as cases had they developed the disease. The controls used in this study were population-based controls. As the cases represent affected individuals in

a pre-defined population, using this control selection method assures a good level of comparability. The controls came from the same source population as the cases.

Matching in a case control study design is a technique that is employed to control for confounding. During matching, subjects are selected in such a way that the potential confounders are distributed in an identical manner among the case and control group. When combined with appropriate statistical analysis, control of confounding by the matching factors is achieved. This can be time-consuming and expensive if very large numbers of subjects have to be closely matched for several variables, but with achievable numbers of subjects, and matching on only a small number of variables (eg. Sex and age) it can be a useful study tool.

As this study was focused on risk factors in teenagers and risk of developing disease is dependant on age, controls had to be age-matched. Male sex is a risk factor for disease [^{43,236}] and girls are likley to be over-represented amongst controls, so sex-matching was also important. Socioeconomic status is a known risk factor for disease and not a hypothesis of this study so implicit socio-economic status matching was assumed by recruiting from the GP of the index case. As numbers were comparatively large and adequate power could be achieved with a single control this was the method chosen.

After recruitment of a case to the study, the GP of the case was contacted for assistance in recruiting a suitable control (see appendix 6). In order to maintain patient confidentiality and comply with the Data Protection Act of 1998 [²³⁷], the GP was required to approach all possible controls before they were contacted by the study team. To this end a recruitment pack was sent out to the participating GP containing information about the study for the GP, along with documentation to be sent out to selected controls (see appendix 17). The documentation consisted of an information sheet for the controls (see appendix 11), a covering letter for the GP to sign (see appendix 18) and a response form for the control to return (see appendix 20). In addition, the GP was invited to return to the study centre a list of the controls he or she had selected along with their contact details (see appendix 19). This was confidential patient information and as such some GP's were unhappy to release it. In these circumstances we had to rely on the returned response forms alone when recruiting a control.

Each GP was asked to send this information to the four patients on their list who were of the same sex and had dates-of-birth closest to that of the index case. Each control pack was numbered one to four. Each Response Form also carried a number one to four. These numbers corresponded to numbers one to four on the Control Identification Form that the GP returned to the study centre. In this manner it was possible to identify the first through to the fourth control selected. If the numbering on the Control Identification Form did not correspond with the numbers on the Response Forms returned by controls, the Control Identification Form was chosen as the correct listing. This was because a completed Control Identification Form was more often returned to the study centre than all four Response Forms.

Selection bias can occur when inclusion of cases and controls depends in some way on the exposure or exposures being studied. Those that agree to take part may in some way be systematically different from those who do not. Four potential controls, randomly selected by date of birth, were sent information about the study and invited to participate. The first numbered control was selected where possible. If the response rate from the first numbered control was low, and a large percentage of selected controls come from numbers two to four, this would result in selection bias. In addition, if the first control that returned a positive response form was recruited this could also result in selection bias. It was therefore important to achieve as high a recruitment rate of first numbered controls as possible.

However, given the constraints of the Data Protection Act this was difficult to achieve. Once we had confirmed that the packs had been sent out to four potential controls by the GP, we would request the Control Identification Form. If the GP was not happy to provide this, then this was respected. Any returned Response Forms were filed until we received a returned form from control number one. If we did not receive a form from control one within two to three weeks of the GP sending out the packs, a reminder letter was sent to this control from the study centre using the data provided to us on the Control Identification Form. (If the GP had not provided us with this form, the control would not be pursued further and the highest ranked control returning a positive Response Form would be recruited.) If there were still no response from control one, they would be telephoned. Permission to do this was given by the GP and made explicitly clear on the original letter from the GP to the

control (see appendix 18). If after these attempts we could not recruit control one we would attempt to recruit control two and so on. If at any stage we received a negative Response Form, no contact would be made.

If we received refusals from all four controls, or if the GP would not provide a Control Identification Form and we had no Response Forms returned, we would ask the GP to send out packs to four more potential controls.

Once a control was selected, a date was arranged for a home visit.

6.5 Interviewing subjects

Apart from specimens collected from case subjects on admission to hospital, all data were collected from subjects during the home visit. Wherever possible the visit to a case and matched control were undertaken on the same day to reduce travel costs. This was not possible when recruitment of a control was difficult, as the length of time between the cases' admission to hospital and interview became too long. A separate visit to the control (at a later date) then became necessary in order to minimise recall bias on the part of the case subject.

Interviews were undertaken by the Research Fellow (JT) and by four trained Research Nurses (JR, JS, ES, JB). Interviewers were not blind to case-control status. On arrival at their home, all subjects were asked to sign a consent form (see appendices 13 and 14) and had the study explained to them. The questionnaire was completed using a standard pre-agreed format. The standard format of the questionnaire left little room for subjective interpretation by the interviewer, and minimised any observation bias resulting from the non-blinded status of interviewers. In order to minimise inter-observer variability in the way in which the questionnaire was delivered, interviewees were trained in the techniques required during interview and attended interviews as an observer prior to conducting them on their own. Memory aides were employed to assist subjects in recall of events. Personal diaries were referred to where relevant, as were diaries used for school or college. Timelines for the relevant fortnight period were constructed for each subject at interview in order to stimulate memory for events.

The interviewer recorded subjects' responses on the questionnaire, except for Sections J and K which the subjects completed themselves (see appendix 24).

The accuracy of self-reporting of behaviour by adolescents has been questioned [²³⁸]. The specific behaviours referred to in the questionnaire might be ones that teenagers perceive to be socially unacceptable, especially to parents or to health professionals (for example smoking, illegal drug-taking, intimate kissing, binge consumption of alcohol). This could lead to the phenomenon of social desirability bias – the tendency of an individual to convey an image in keeping with social norms and to avoid criticism [²³⁹]. In an attempt to overcome this, all interviews were conducted separately from the subjects' parents. This was made explicit in all information sheets sent to subjects. On a few occasions a parent requested to be present during the interview. This was deemed acceptable if the subject was in agreement.

A sample of blood was taken from the case subjects at interview. Samples of blood, a throat swab and a pernasal swab were collected from control subjects at interview.

6.6 Laboratory specimens

Laboratory specimens were collected to test the hypothesis that there is a relationship between respiratory tract infection and invasive MD. An association was also postulated between Mannose Binding Lectin deficiency and invasive infection. Meningococcal serology serves as one diagnostic method to confirm infection.

Specimens were collected from study subjects relating to the above.

6.6.1 Collection from subjects

6.6.1.1 Case subjects

Case subjects had samples collected on admission to hospital (the acute samples) and at interview (the convalescent samples). The acute samples were a single sample of blood (serum), a throat swab and a pernasal swab. The serum sample was tested for Influenza A and B virus serology (acute titre) and meningococcal serology (acute titre). The swabs were submitted for PCR analysis for Epstein Barr Virus, Respiratory Syncytial Virus, Chlamydia and Mycoplasma.

The hypothesis was that respiratory viral infection might form one part of the causal chain leading to invasive meningococcal infection. This means that viral infection has to occur before meningococcal infection. Once infected with a virus the host immune defences will be activated to clear the invading organism. It is possible that the predisposing infecting organism may have been cleared by the host by the time meningococcal infection becomes clinically apparent. Therefore, in order to maximise the chances of isolating viruses or organisms by PCR (a technique that requires pathogen DNA to be present in host tissue), it was important to collect the swab specimens as soon as possible after the case subject was admitted to hospital. An arbitrary 5-day cut-off period was applied. If swab specimens could not be collected from the case subject by the fifth day of their admission, they were not collected. This was done so that analysis would not be confused by the results from swab specimens collected at a late stage when virus isolation would not realistically be a possibility.

Once a patient or family had consented to involvement in the study, a study pack was delivered by courier to the ward. This contained all the equipment required for collection of the acute samples (see appendix 16), along with return courier packaging. The courier pack arrived on the ward the day after consent had been obtained. Samples were collected by the attending clinician and sent to the Central Public Health Laboratory (CPHL) at Colindale by return courier where all the specimens were collated.

The case convalescent sample was collected at interview. The convalescent sample consisted of two blood samples (serum and whole blood) for Influenza A and B virus serology (convalescent titre), meningococcal serology (convalescent titre), Epstein Barr Virus serology (convalescent titre) and Mannose Binding Lectin genetic analysis (whole blood).

Samples were sent by special postal delivery to the CPHL.

6.6.1.2 Control subjects

Control subjects had specimens collected only at interview. It would not have been logistically possible to identify, obtain consent from and visit a control subject in

time to collect acute samples as were collected from cases. In temporal terms these therefore constituted the convalescent sample.

Two samples of blood (serum and whole blood) were taken for Influenza A and B titres (convalescent), Epstein Barr Virus titres (convalescent) and Mannose Binding Lectin genetic analysis. Throat and pernasal swabs were taken for PCR for influenza A and B, RSV, chlamydia and mycoplasma.

All were sent by special postal delivery to the CPHL where samples were to be processed and analysed.

6.6.2 Specimen handling

Blood was stored at +4 degrees celsius between collection and transport. Swabs were placed into Public Health Laboratory Services viral transport medium and stored at +4 degrees celsius. Time between collection of specimens and arrival at the central laboratory was minimised by the use of couriers and special postal services.

All specimens were sent to the CPHL where the whole blood sample was frozen at – 20 degrees Celsius. The other sample was spun and separated, and the serum divided into four aliquots. These were also frozen at –20 degrees Celsius. The viral medium containing the swab samples was frozen at –70 degrees Celsius.

Once data collection was complete and all samples had been collated at CPHL, they were redistributed to the laboratories responsible for final analysis.

The aliquots of serum from both cases and controls were thawed and sent to the appropriate laboratories by courier. A set of convalescent serum for cases and controls went to Professor D Crawford, Medical Microbiology Department, University of Edinburgh for Epstein Barr Virus serology analysis. Aliquots of acute and convalescent serum on case subjects were sent to the laboratory of Dr E Kaczmarek at the Meningococcal Reference Laboratory in Manchester for meningococcal serology analysis.

Whole blood samples were thawed and sent by courier to the laboratory of Dr Nigel Klein at the Institute of Child Health, London for MBL genetic analysis.

Aliquots of serum and swab sample material for respiratory viral studies were sent to Dr M Zambon in the Respiratory Viral Division of the CPHL, while further swab sample material was sent to Dr R George in the Respiratory and Systemic Infections Division at the CPHL for Chlamydia and Mycoplasma PCR.

6.6.3 Respiratory viral studies

If respiratory viral infection predisposes to invasive meningococcal infection, the length of time between viral infection and clearance by the host immune system, and development of clinically apparent MD is unknown. Predisposing viruses may have been cleared by the host by the time meningococcal infection becomes clinically apparent. This would make it difficult to confirm respiratory viral infection in a subject, even if it had occurred [³⁸].

The respiratory pathogens selected for investigation were those that cause respiratory tract inflammation and/or a degree of suppression of the host immune system functioning, and that exhibit similar seasonality to meningococcal infection. There also had to be reliable methods for diagnosing recent infection. The respiratory pathogens selected were;

Influenza A (subtypes H3N2 and H1N1) and influenza B

Epstein Barr Virus

Respiratory Syncytial Virus

Chlamydia

Mycoplasma

One or both of two methods were employed to attempt to confirm infection in study subjects. The first of these used serological techniques and the second used polymerase chain reaction (PCR).

6.6.3.1 Influenza Virus

For the case subjects, both acute and convalescent serum samples were analysed, along with the single “convalescent” serum sample for the control. Swab material

was also analysed, case swabs being taken at the time of admission to hospital (acute) and the control swabs at interview (convalescent).

Serum samples were used to measure anti-influenza antibody production. This was determined using the haemagglutinin inhibition antibody (HIA) test. HIA tests were performed for viral strains A/Bayern/7/95 (H1N1), B/Jarbin/7/94 (type B) and A/Sydney/5/97 (H3N2). The H3N2 virus was selected as it had circulated during the course of this study. The B/Harbin (type B) and A/Bayern (H1N1) strains were not circulating to any significant degree and could act as comparative standards. Where sufficient sera were available, each test was performed in duplicate with all antigens and an arithmetic mean titre was calculated. HIA titres of >320 against influenza A H3N2 and >80 for influenza B were taken to indicate infection within the last year (personal communication Dr M Zambon). Samples with titres < 10 units were recorded as negative. Missing data were recorded where this was the case for both runs.

Throat and pernasal swabs were used for reverse transcriptase PCR (RT-PCR) analysis. This technique amplifies specific viral DNA and is an accurate method of diagnosing recent infection because it relies on the presence of the virus within host tissue.

6.6.3.2 Epstein Barr Virus

A single titre measurement was performed on convalescent samples from cases and controls for EBV infection. VCA IgG on the samples indicates if the subject had ever been infected, while VCA IgM indicates more recent infection. If a subject was VCA IgM positive, a monospot was performed to confirm recent infection.

PCR work was not performed on samples for EBV.

6.6.3.3 Respiratory Syncytial Virus

PCR for RSV was performed on the throat and pernasal swabs from case and control subjects.

6.6.3.4 Chlamydia and Mycoplasma

PCR for chlamydia and mycoplasma was performed on the throat and pernasal swabs from case and control subjects.

6.6.4 Mannose Binding Lectin

Mannose binding lectin forms part of the innate immune system. Seven distinct haplotypes along with promoter region polymorphisms regulate serum levels of MBL. Three single nucleotide substitutions in exon 1 of the gene lead either to low or very low levels. An individual's genetic make-up with regard to Mannose Binding Lectin production was determined using DNA from whole blood. Samples were collected from all study subjects at time of interview.

6.6.5 Meningococcal serology

As recruitment of case subjects depended on a clinical diagnosis of meningococcal infection and laboratory diagnosis of infection is often not possible early in the course of disease (at the stage recruitment occurred), a decision was made to analyse data in two groups. One group comprised those subjects on whom we achieved laboratory confirmation of disease and the other group included those on whom there was no laboratory confirmation.

Serological diagnosis of meningococcal infection is one method employed to confirm infection. It relies on a paired blood sample being taken, one in the acute stage of disease and one at convalescence. As this is often not performed routinely at admitting hospitals, the convalescent sample was collected for serological diagnosis from case subjects at interview. Along with routine tests performed by admitting clinicians, this would provide a laboratory-confirmed diagnosis in as many subjects as possible.

6.6.6 Temporal difference in sample collection

The respiratory viral infections investigated in this study are primarily winter infections and have a seasonality that coincides with the peak occurrence of MD. In order to demonstrate a difference in the respiratory viral infection rate between cases and controls that is meaningful in relation to the development of invasive MD,

samples taken for the diagnosis of viral infection should ideally be taken at the same time in both cases and controls.

The methodological difficulty with the design of this study was that, for logistic reasons already discussed, an acute set of samples could not be taken from the control subject at the time acute samples were taken from the case.

For the purposes of matched analysis, comparisons were made between the convalescent blood samples from the case and control. In the great majority of subject pairs the case and control were interviewed on the same day, therefore these samples were taken on the same day and represented the same convalescent period.

The acute blood sample taken from the case subject on admission was used as a baseline for observing a possible rise in serological titres; it could not be compared with an equivalent control acute sample.

The results from the case and control swabs were compared. However, there was a temporal discrepancy between the time at which the case subject swabs were collected (on admission to hospital) and the time at which the control swabs were collected (at interview). Although both sets of swabs were being collected acutely in terms of the period of time about which the subjects were being questioned at interview, this was not always the same period of time in terms of seasonality and background risk of respiratory and meningococcal infection, and the possible difference in other related risk factors. It was important therefore to keep the length of time between admission of the case subject to hospital and interview of case and control subjects to a minimum.

In an attempt to overcome this possible methodological criticism, a question was asked of all control subjects at interview. This question asked the control whether they had been unwell in the week leading up to the date of their matched case subjects' admission to hospital. The possibility of significant recall bias must be considered here as the length of time between case admission to hospital and control interview was sometimes high.

During statistical analysis it was therefore necessary to find a method of controlling for season so that any possible discrepancy between cases' and controls' time frames

would not confound results. A 'high' influenza season is defined as a consultation rate >50 per 100,000 of population per week as detected via the sentinel general practice surveillance network of the Royal College of General Practitioners in England and Wales [²⁴⁰]. The 'medium' influenza season is defined as the period of time between the first hospital case identified and the last community case identified (personal communication M Nunn). During the period of this study there were two clearly defined 'high' seasons for influenza A, running from week 51 of 1998 to week 5 of 1999 and from week 50 of 1999 to week 4 of 2000. The two 'medium' seasons ran from week 41 of 1998 to week 14 of 1999 and from week 40 of 1999 to week 8 of 2000 respectively. The remaining time intervals were classified as belonging to the 'low' influenza season. For all subjects it was recorded whether the time to which data pertain was in the high or low risk season. For cases this was the time of infection, for controls the time of interview.

6.7 Data handling and analysis

6.7.1 Formic Automatic Data Capture

Questionnaire data were entered onto the computer using the Formic 3 form design and automatic data capture system for Windows [²⁴¹]. This is a rapid and accurate method of entering large amounts of data. The accuracy of data entry achieved is superior to that using manual double data entry. Data are processed and automatically entered into a Formic database.

6.7.2 Data checking and database design

Once data collection was complete the data were exported from the Formic database into Microsoft Excel 97 [²⁴²]. Each subject's data were then checked for internal inconsistencies and missing data points. Where possible, missing data points were completed by contacting the subject, family or admitting hospital. Internal inconsistencies were dealt with by referring back to the original questionnaire data.

A database was constructed within Excel 97 containing all raw data. This was converted into a suitable form for Stata 6 and all data exported into this program for statistical analysis.

6.7.3 Statistical analysis of data

All analyses were carried out in STATA 6.0 [²⁴³]. Data were entered into Stata as categorical or continuous variables. The classification of all categorical variables was decided prior to analysis. Descriptive statistics were applied to assess matching and identify differences between characteristics of cases and controls. Any association between MD status, potential confounders and risk factors were assessed by means of univariate contingency tables and thereafter by multivariate conditional logistic regression. Multivariate conditional logistic regression analysis is a statistical technique that takes into account a number of variables simultaneously. It allows for the estimation of measures of association while controlling for a number of confounding factors at the same time. A regression model was constructed that described most efficiently the relationship between exposures and disease, taking into consideration possible confounding variables. Predictor variables were included in the logistic regression model if they were a major or minor hypothesis or contributed to the model fit with a p-value <0.2 [²⁴⁴]. Socio-economic status variables (car/home ownership and subject occupation) were retained in the model regardless of their significance.

Both MD and respiratory illness show seasonal variation. Controls were recruited with a time lag and therefore potentially within a different season from the case. Controlling for the effect of time lag and different seasonality between the interview of the case and that of the matched control was achieved by introducing a 'high/low MD season' covariate ("season") in the logistic regression model as previously described [²⁴⁵].

6.8 Difficulties encountered with the study

6.8.1 Subject recruitment

Ideally, recruitment of cases to a study should approach 100% of the number of cases that occurred in the area covered by the study during the period the study was running. Although recruitment rates in this study were acceptable, they did not reach 100%. The logistic constraints placed by recruiting subjects from a large area of the country, the need to recruit soon after admission coupled with reliance on other

health professionals for referral of possible subjects, meant that some subjects were inevitably missed.

6.8.1.1 CCDC non-collaboration

Consultants in Communicable Disease Control have many demands made upon them from research teams. Requests for data are frequently made. For this reason some CCDC's were not happy to participate in the study and declined to notify the study centre of possible subjects for inclusion. In particular, all CCDC's from one region declined to participate. Thus in this region data from local clinicians and the Manchester Reference Laboratory became increasingly important.

In order to provide feedback to participating CCDC's and to maximise notification throughout the study period, a quarterly circular was sent out containing information about the study progress. In addition, computer mousemats carrying a study logo and contact telephone numbers were designed. These were sent to all CCDC's and to Intensive Care Units in the regions included.

6.8.1.2 GP non-collaboration

In a few cases, the GP of the index case declined involvement in the study. This was usually because the GP was uncomfortable disclosing patient details. In these cases an agreement was made that no patient details would be released to us and only the control that returned a positive response form would be recruited.

If a GP declined involvement completely, the Family Health Services Association was contacted for the nearest alternative GP practice in the area, and they were approached to request collaboration.

6.8.1.3 Late referrals

In order to collect the blood and swab specimens required, an initial decision was made to recruit cases only if the specimens could be collected by the fifth day of admission. However, it soon became apparent that many potential subjects were not being recruited because referral to the study centre was too late. The study protocol was then altered to allow recruitment of subjects at any stage after admission, but collection of swab specimens was only done if it could be achieved by the fifth day of admission. If this could not be achieved "acute" sample collection was omitted.

In certain cases, referral reached the study centre after the subject had been discharged from hospital. If a diagnosis of MD had been confirmed by this stage, recruitment was attempted through the subject's GP and no acute specimens were collected.

6.8.2 Subject numbers

Our initial power calculations estimated that 163 case-control pairs would need to be recruited to the study. By the time data collection finished, 144 case control pairs had been recruited. However, the initial power calculations were done using an unmatched design, so once matching had been achieved the actual numbers required were less as matching serves to increase the power of a study.

6.8.2.1 The effect of introduction of the vaccine

In the Autumn of 2000, the Department of Health introduced a new conjugate vaccine against serogroup C MD into the UK. Teenagers aged 15 to 17 years were among the first group to receive this vaccine. In addition college students entering their first year were vaccinated with the "old" bivalent A/C polysaccharide vaccine. As nearly 50% of teenagers who contract MD have serogroup C infection, this would almost certainly affect the numbers available for recruitment during the second winter period of the study. By the time data collection ceased in June 2000 (8 months after the vaccination programme started), the conjugate vaccine had indeed reduced the number of cases of serogroup C disease in the groups targeted for vaccination by 75% [²⁴⁶]. This meant that fewer cases occurred during the winter of 1999/2000 than originally anticipated and this had a small effect on final numbers recruited.

6.9 Bias and case control studies

Bias can be defined as any systematic error in a study that results in an incorrect estimate of the association between exposure and risk of disease [²⁴⁷]. Since the effects of bias are difficult to take into account during analysis, it is important that study design minimizes the introduction of bias and interpretation of results recognises and acknowledges bias and the direction and magnitude of possible

effects. Bias is of particular importance in case-control studies due to the inherent nature of this type of investigation.

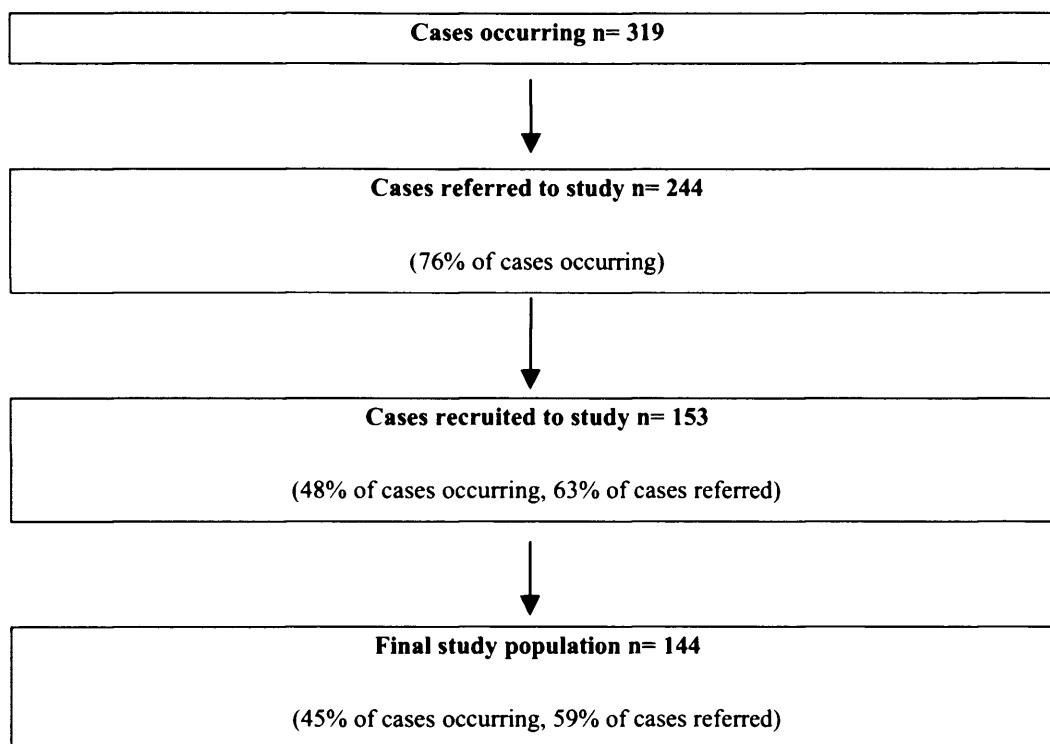
6.9.1 Selection bias

Selection bias can occur when the inclusion of cases and controls into the study depends on the exposure or exposures of interest [²⁴⁸]. In case-control studies this can be a very real problem since both exposure and disease have already occurred at the time of selection. Selection bias will result in systematic differences between the case and control groups that may be related to the exposures under investigation. Concerns that selection bias may exist are especially raised if response or recruitment rates of cases and controls are low or unequal [²⁴⁸].

6.9.1.1 Case selection

During the study period there were 319 cases of meningococcal disease in 15 to 19 year olds reported throughout our six regions of England. This is the total population eligible for inclusion. Of these a percentage were referred to the study centre for consideration for entry into the study (see figure 3). This percentage can be assumed to be close to randomly selected as referral did not depend on characteristics of the case, but on characteristics of the referrer. Of cases referred, a certain number were successfully recruited, others were not recruited for a variety of reasons, some declined involvement or died prior to recruitment, and a further proportion were lost to follow-up after recruitment (see section 7.1.1.1).

Figure 3 Case recruitment patterns



The reasons why referred cases were not recruited are important in ascertaining the likelihood of recall bias. A small percentage of non-recruitment was due to subject refusal or losses to follow-up (see section 7.1.1.1). These particular cases may be systematically different from the cases that consent (eg. In socio-economic status) and therefore have systematic differences in the exposures under investigation (eg. smoking). This percentage was acceptably small.

Subjects who died (by definition the most severe cases) were not recruited. In terms of bias this may introduce, if the hypothesis states that exposures lead to disease, then excluding the most severe cases will, if anything, underestimate the effect of risk exposures under study.

The reasons why the remaining non-recruited cases (that form the majority) were not included are various and will be randomly distributed so are unlikely to lead to selection bias.

6.9.1.2 Control selection

Selection bias in case control studies can derive from refusal or non-response among potential participants. Although low response rates do not necessarily indicate the presence of bias, if the response rate also relates to the exposure status then bias will be introduced. This was an important consideration during control selection as individuals who agreed to take part and who are motivated to respond, may be systematically different from those who do not. This may be in a way that is related to the exposure under investigation (eg. Social class, educational level etc).

Controls were recruited via the GP of the index case. The aim was to recruit as many first-numbered controls as possible and where this was not possible to be systematic and rigorous in our method of selecting a control (see section 6.4.2). In this way, although selection bias remains a possibility, every step possible was taken within the constraints of study design and data protection restrictions, to guard against it. Of 144 controls recruited to the study, 55 (38%) were the first numbered control and 36 (a further 25%) the second selected control. In 20% of cases further potential controls had to be approached as the first three selected controls did not respond.

6.9.2 Observation or information bias

This type of bias results from systematic differences in the way data are obtained from the two groups [²⁴⁷]. If the inaccuracy or incompleteness of data collection affects the two groups unequally observational or information bias may result.

There are different types of observation or information bias;

6.9.2.1 Interviewer bias

This refers to any systematic difference in the recording or interpretation of information from cases and controls [²⁴⁷]. This can be a particular problem in case-control studies because the disease status of the case is already known. The only method of ensuring against this type of bias is by interviewer blinding.

In this study the interviewers were not blinded to case-control status. This was because certain questions were directed only at cases and others only at controls, so blinding was not possible. However the possibility of interviewer bias was taken into account during study design. The structured questionnaire used left little room

for interpretation. In addition all four research nurse interviewers underwent a period of training and observation by the Clinical Research Fellow (JT) to ensure standardisation in the way in which responses were elicited.

6.9.2.2 Recall bias

This type of bias is particularly problematic in case-control studies. It arises when exposed subjects recall or report events leading up to the exposure differently from unexposed subjects. Recall bias can lead to either an over- or under-estimate of the true association between exposure and disease [²⁴⁷].

6.9.2.2.1 Recall bias in cases

Case subjects were questioned about the 2-week period preceding their admission to hospital with MD. A period of time had elapsed between admission to hospital and interview. In order to minimise bias every effort was made to interview case subjects as quickly as possible after discharge from hospital. This was a median of 53 days after admission.

6.9.2.2.2 Recall bias in controls

Ideally the control subjects in this study would be questioned about exactly the same fortnight period as the case subjects. However, as there was a period of time between admission of the case and recruitment and interview of the control, a decision was taken to interview control subjects about the fortnight immediately preceding interview. Although this was a different time period than the fortnight the cases were questioned about, the controls would be unable to recall events with sufficient accuracy or in sufficient detail if an “arbitrary” fortnight period was chosen.

Although every effort was made to minimise the possibility of significant recall bias occurring in this study, its effects must not be ignored during interpretation of the data. It is difficult to estimate the magnitude and direction of effect that any recall bias may have.

6.9.2.3 Social desirability bias

Social desirability bias is a phenomena whereby subjects report exposures in a manner which they perceive to be acceptable to the interviewer. Collection of data from adolescents about social behaviours (in particular smoking habits) has been criticised for being heavily subject to bias [²³⁸]. However, others have suggested adolescent self-reporting of behaviour can be accurate. [^{94;96}]. In this study the figures relating to smoking behaviour were in line with nationally reported figures.

6.9.2.4 Other possible bias

Information was elicited from subjects relating to behaviours in the fortnight prior to admission to hospital (cases) or interview (controls). In addition we asked about preceding (cases and controls) or prodromal (cases only) illness. A possible source of bias could be that subjects experiencing preceding or prodromal illness during this fortnight period, might change their normal habitual behaviours due to ill-health. An example might be a subject with a preceding illness not attending church that week or a subject with the prodrome of meningococcal disease drastically reducing their cigarette and alcohol consumption because they feel unwell. This would introduce bias into the results. In an attempt to overcome this problem, data were collected after completion of this study regarding habitual behaviour in respect of church attendance and smoking behaviours over the prior year (personal communication Jennie Borg).

7 Results

7.1 Recruitment

7.1.1 Case recruitment

During the study period 319 statutory notifications of MD in teenagers aged 15 – 19 years were made to public health units in the study regions. Of these, 244 were referred to the study centre and 153 were recruited to the study. This gives a referral rate of 76%, a recruitment rate of 48% of total cases and a recruitment rate of 59% of referred cases.

7.1.1.1 Reasons for non-recruitment

There were 91 cases referred who were not recruited to the study. The reasons for this were as follows;

- 23 were referred after the 5th day of admission,
- 18 came from districts where local ethical approval was delayed,
- 16 died before recruitment,
- 12 had an alternative diagnosis,
- 11 subjects refused,
- 5 the clinician in charge refused,
- 3 did not speak English,
- 3 were not recruited due to staffing shortages.

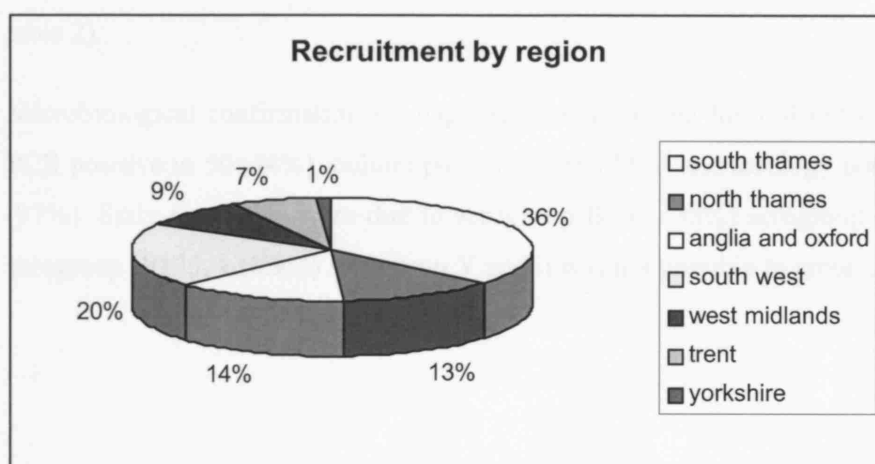
Of the 153 recruited, 2 died after recruitment from complications of severe meningococcal infection, 2 later refused to participate and 5 were lost to follow-up, resulting in 144 cases from which questionnaire data were collected.

7.1.1.2 Source of recruitment

Referrals to the study came from four sources (see section 6.2.2). Eleven cases were referred for inclusion from more than one source. CCDC's referred 55% of cases, the Manchester Reference Laboratory referred 24% of cases, 12% were referred by hospital staff and 9% were referred from the Meningitis Research Foundation.

Subjects were recruited from six regions of England plus a single Health Authority in Yorkshire.

Figure 4 Recruitment by region



7.1.2 Control recruitment

Controls were recruited using the General Practitioner of the index case.

Of the 144 controls recruited to the study, 55 (38%) were the first selected control and 36 (a further 25%) were the second selected control. For 28 cases (20%) we were unable to recruit one of the first 3 selected controls and had to approach further potential controls (see section 6.4.2).

7.2 Demographic characteristics of subjects

Of the 144 case-control pairs, 74 (51%) were male. The median age at referral was 17.6 years for cases and 17.7 years for controls. One hundred and twenty-eight (89%) cases and 134 (93%) controls described themselves as being of white

ethnicity. The median time from admission of the case to interview was 53 days (range 4 - 343) for cases and 64 days for controls (range 19 - 317). The majority of study subjects (71% of cases and 67% of controls) were either school or university/college students, and most lived at home with family. The majority of subjects were of high socioeconomic status (SES) using car and home ownership as indicators. There were slightly more “professional” and “intermediate” head of households amongst the control group. Slightly more control head of households than case head of households also owned a car and their own home but this difference was not significant at univariate analysis. Cases and controls were generally well matched for SES, living arrangement and employment status (see table 2).

Microbiological confirmation of diagnosis was available for 114 out of 144 cases; PCR positive in 50 (44%), culture positive in 38 (33%) and serology positive in 111 (97%). Sixty-six (58%) were due to serogroup B, 43 (38%) serogroup C, 1 (0.9%) serogroup W135, 1 (0.9%) serogroup Y and it was not possible to group 3 strains.

Table 2. Demographic data on participating cases and controls

	Category	Cases n=144 n (%)	Controls n=144 n (%)	Matched OR (95% CI)	P value
Age (years)	15 16 17 18 19	25 (17) 28 (19) 30 (21) 28 (19) 33 (23)	23 (16) 23 (16) 34 (24) 27 (19) 37 (26)		
Ethnicity	White Black Indian Asian Others	128 (89) 5 (3) 6 (4) 5 (3)	134 (93) 2 (1) 3 (2) 5 (3)	1 1.3 (0.2 – 10.2) 3.1 (0.6 – 15.8) 1.3 (0.3 – 6.0)	0.44 0.79 0.17 0.71
Socioeconomic status (of head of household) by home and car ownership	Not house or car Not house, ≥1 car Own house, no car Own house, ≥1 car Missing data	12 (8) 22 (15) 7 (5) 102 (71) 1	12 (8) 16 (11) 5 (4) 110 (76) 1	1 1.4 (0.5 – 4.4) 1.4 (0.3 – 6.6) 0.9 (0.4 – 2.3)	0.67 0.53 0.64 0.83
Socioeconomic status (of head of household) by Registrar General Classification	Professional Intermediate Skilled, non-manual Skilled, manual Partly skilled Unskilled Unclassifiable by Reg General classification Missing data	11 (8) 40 (29) 18 (13) 36 (26) 10 (7) 1 (0.7) 22 (16) 6	19 (14) 48 (35) 15 (11) 29 (21) 12 (9) 2 (1.5) 12 (9) 7	1 1.4 (0.6 – 3.2) 2.2 (0.8 – 6.2) 2.1 (0.9 – 5.2) 1.4 (0.5 – 4.5) 1.1 (0.1 – 15.1) 4.4 (1.3 – 14.8)	0.29 0.43 0.15 0.09 0.54 0.92 0.017
Employment status (cases & controls)	Employed School student University student Unemployed/Home duties	36 (25) 52 (36) 50 (35) 6 (4)	38 (26) 51 (35) 46 (32) 9 (6)	1 1.1 (0.4 – 2.8) 1.2 (0.6 – 2.4) 0.6 (0.2 – 2.5)	0.83 0.82 0.66 0.52
Living arrangements (cases & controls)	With family only With friends only With partner and/or friends or family Missing data	121 (84) 18 (13) 4 (3) 1 (1)	123 (85) 16 (11) 4 (3) 1 (1)	0.9 (0.4 – 1.9) 1 (0.5 – 2.2) 1 (0.3 – 4.0)	0.7 1 1

OR indicates Odds Ratio and CI indicates Confidence Interval

7.3 Univariate analysis results

Univariate matched analysis was performed for all major variables. The following results are presented in three sections relating to the three major hypothesis areas of this study; biological factors, social factors and psychological factors.

7.3.1 Biological hypotheses

A major hypothesis was that preceding infection, particularly with respiratory viruses, might predispose to invasive meningococcal disease. In addition, it was hypothesised that MBL deficiency might predispose to disease.

7.3.1.1 Preceding illness

In the univariate matched analysis, cases were significantly more likely to report being unwell with a preceding illness in the fortnight prior to admission than were the controls in the fortnight prior to interview ($p=0.001$) (see table 3). Because cases and controls were unavoidably interviewed about different periods of time (the 2 weeks prior to admission for cases and the 2 weeks prior to interview for the controls), and these 2 time periods might fall in different seasons, a seasonal adjustment was performed. The effect of reported preceding illness remained significant when season was taken into account ($p=0.006$). This is reassuring despite the fact that 72% of controls were interviewed in the same season as the case was admitted to hospital. More controls reported having used antibiotics in the fortnight prior to interview than cases in the fortnight prior to admission (11 controls versus 5 cases).

Samples for viral serology were available for 105 case-control pairs (73%). There were no differences between cases and controls in the proportions with Influenza A H3N2 or H1N1 titres > 320 or Influenza B Titres > 80 . Of 81 cases with paired serology, 9 (11%) showed at least a four-fold rise for influenza A (H3N2 7, H1N1 2) and 5 (6%) showed at least a four-fold rise for influenza B. More cases than controls were positive for EBV VCA IgG ($p=0.12$); 3 of 129 (2%) cases and none of 116 controls were EBV VCA IgM positive.

Throat or pernasal swab specimens were collected from 86 cases and 139 controls. There were no PCR positive results from throat or pernasal swabs for influenza A or B, chlamydia, mycoplasma or RSV.

7.3.1.2 Mannose-binding lectin

Samples for MBL analysis were available for 92 case-control pairs (64%). Data were analysed in three ways – according to the level of MBL production, using the structural gene composition (mutations in exon 1 of the gene) and by examining promoter region polymorphisms (see table 4).

Very low production of MBL was not associated with disease.

When data were analysed using the structural gene composition, there was no difference between case and control subjects. Most subjects were either wild type (49% cases & 55% controls) or heterozygote (45% cases & 43% controls) for structural gene mutations. Only 4% of cases and 1% controls were compound heterozygote recessive while the homozygote recessive group were the minority (1.8% of cases and 1.7% of controls).

When examining promoter region polymorphisms, there were again no differences between cases and controls. Sixty-seven percent of cases and 65% of controls displayed the wild type promoter region, 32% of cases and 33% of controls were heterozygote for the promoter region and less than 1% of cases and 2% of controls were promoter region homozygote recessive.

None of the results for MBL reached significance at univariate analysis.

7.3.1.3 Other biological factors

Twenty-six percent of cases and 38% of controls recalled having been vaccinated against meningococcal disease prior to enrollment in the study. This vaccination data included both the polysaccharide and conjugate vaccine. Previous immunization against meningococcal infection was found to be protective (see table 3) .

Table 3. Univariate analysis for biological factors excluding MBL

Variable	Cases n (%)	Controls n (%)	Matched OR (95% CI)	P value
Preceding illness*	76 / 144 (53)	45 / 144 (31)	2.3 (1.4-3.7)	0.001
Preceding illness (controlled for 'high' MD season)	-	-	2.0 (1.2-3.3)	0.006
Antibiotic usage*	5 / 144 (4)	11 / 139 (8)	0.45 (0.2-1.3)	0.13
Influenza H3N2 convalescent titre >320	48 / 116 (41)	45 / 120 (38)	1.2 (0.6-2.2)	0.63
Influenza H1N1 convalescent titre >320	16 / 116 (14)	16 / 120 (13)	0.8 (0.3-2.1)	0.62
Influenza B convalescent titre >80	10 / 116 (9)	8 / 120 (7)	1.2 (0.4-3.5)	0.78
EBV (VCA IgG positive)	105 / 129 (81)	89 / 116 (77)	1.8 (0.9-3.6)	0.12
EBV (VCA IgM positive)	3/129 (2)	0/116 (0)	-	-
Received meningococcal vaccination (polysaccharide or conjugate) prior to date of disease	37 / 144 (26)	54 / 143 (38)	0.4 (0.2-0.8)	0.01

OR indicates Odds Ratio and CI indicates Confidence Interval

* in fortnight period before illness (cases) or interview (controls)

Table 4. Univariate analysis for Mannose Binding Lectin

Variable	Cases n (%)	Controls n (%)	Matched OR (95% CI)	p-value
MBL (Very low producers)	20/112 (18)	13/111 (12)	1.3 (0.6-3.0)	0.53
MBL (Structural gene composition)				
<i>Wild type</i>	56/114 (49)	63/115 (55)	1.0	0.39
<i>Heterozygote</i>	51/114 (45%)	49/115 (43%)	1.1 (0.6-2.0)	0.69
<i>Compound heterozygote</i>	5/114 (4)	1/115 (0.9)	5.6 (0.6 – 274.6)	0.08
<i>Homozygote recessive</i>	2/114 (2)	2/115 (2)	0.5 (0.05-5.8)	0.6
MBL (promoter region polymorphisms)				
<i>Wild type</i>	76/113 (67)	72/111 (65)	1.0	0.87
<i>Heterozygote</i>	36/113 (32)	37/111 (33)	0.9 (0.5-1.7)	0.66
<i>Homozygote recessive</i>	1/113 (1)	2/111 (2)	0.5 (0.04-5.2)	0.53

7.3.2 Social hypotheses

The main social hypotheses were that the risk of MD in adolescence is related to developmental social factors such as mouth kissing, substance use and changing social interaction patterns and that certain leisure activities, including involvement in elite athletic pursuits are associated with an increased risk of MD.

7.3.2.1 Substance use – cigarettes, alcohol and drugs

Almost half of the study subjects reported being a regular cigarette smoker, although most smokers reported smoking less than 10 cigarettes per day. In the univariate matched analysis of substance use variables being a smoker was not associated with disease (see table 5). Many previous studies have linked passive smoke exposure with meningococcal disease, especially in children (see section 4.2). Several variables were included to elucidate passive cigarette smoke exposure (having/not having a partner who smokes, number of household smokers, number of other close contacts who smoke, exposure/non-exposure to smoke at place of work). Of these, reporting multiple close contacts outside the home who smoked was the only one to reach borderline significance at the univariate stage of analysis (see table 5).

Consuming any alcohol in the fortnight prior to admission was associated with disease, as was heavy levels of consumption of alcohol. Both regular use of illicit drugs, use of these drugs in the fortnight prior to admission and sniffing or smoking drugs as opposed to other routes of administration were associated with being a case (see table 5).

Table 5. Univariate analysis for substance use behaviours

Exposures	Cases N=144 n (%)	Controls N=144 n (%)	Matched OR (95%CI)	P value
Regular smoker	47 (33)	45 (31)	1.1 (0.6-1.8)	0.8
Smoked>10 cigarettes per day*	19 (13)	16 (11)	1.2 (0.6-2.6)	0.58
Partner who smokes	27 (19)	18 (13)	1.5 (0.8-2.8)	0.21
1 or more household smokers	79 (55)	69 (49)	1.3 (0.8-2)	0.31
Multiple close contacts who smoke*	104 (72)	92(64)	1.6 (0.9-2.7)	0.11
Exposure to smoke at workplace	62 (43)	51 (35)	1.3 (0.8 – 2.2)	0.28
Any alcohol consumed*	123 (85)	113 (78)	1.6 (0.9-3.0)	0.13
Greater 24units alcohol consumed*	40 (28)	34 (24)	1.9 (0.8-4.3)	0.13
Regular consumption of illegal drugs (once a week or more)	23 (16)	13 (9)	2.3 (1.0-5.2)	0.06
Used Drugs in fortnight period*	24 (17)	16 (11)	1.7 (0.8-3.6)	0.15
Sniffed or smoked drugs*	22 (15)	15 (10)	1.7 (0.8-3.7)	0.18

OR indicates Odds Ratio and CI indicates Confidence Interval

* in fortnight period before illness (cases) or interview (controls)

7.3.2.2 Intimate and social contact and leisure behaviours

In the univariate matched analysis of intimacy variables (see table 6), having more than one intimate kissing contact (deep or mouth kissing) was associated with greater risk. Other intimacy variables (superficial kissing, bed-sharing, having a partner) were not associated with disease.

Univariate analysis of social contact variables revealed that sharing a bedroom appeared to confer greater risk of disease, although living in dormitory accommodation or having overnight guests did not appear to increase risk (see table 6).

In the univariate analysis of the variables associated with social contact and leisure behaviours, several activities were associated with greater risk of disease. These included visiting pubs, bars, nightclubs or parties in the fortnight prior to admission and daily visits to friends' homes (see table 6).

Significantly lower risk of disease was associated with religious ceremony attendance at least once a week (see table 6).

Results obtained for the sports score in the 2-week period prior to admission or interview showed that a moderate amount of exercise (sports score 18-50) was associated with protection against disease (see table 6). Other scores, in particular a score greater than 50 indicating extreme exercise, were not significantly associated with being a case.

Table 6. Univariate analysis for social contact, leisure and intimacy behaviours

Exposures	Cases n=144 n (%)	Controls n=144 n (%)	Matched OR (95%CI)	P value
Regular partner	60 (42)	54 (38)	1.2 (0.7-1.9)	0.45
1 or more superficial kissing contacts*	97 (67)	92 (64)	1.2 (0.7-2)	0.52
Multiple intimate kissing contacts*	42 (29)	22 (15)	2.1 (1.2-3.7)	0.009
1 or more bed-sharing contacts*	44 (31)	42 (30)	1.1 (0.6-1.9)	0.78
Crowding index > 1.5	1 (0.7)	3 (2)	0.3 (0.03-3.2)	0.34
1 or more bedroom-sharing contacts*	96 (67)	74 (51)	2.1 (1.2-3.6)	0.006
Lives in dormitory accommodation	10 (7)	8 (6)	1.4 (0.4-4.4)	0.6
1 or more overnight guests*	43 (30)	34 (24)	1.4 (0.8-2.5)	0.21
Attended pub or bar*	114 (79)	105 (73)	1.5 (0.8-2.7)	0.18
Attended nightclub, disco or party*	97 (67)	81 (56)	1.7 (1-2.8)	0.05
Daily visit to friends home*	31 (22)	15 (10)	2.3 (1.2-4.6)	0.01
Attended youthclub*	9 (6)	13 (9)	0.6 (0.3-1.6)	0.35
Visited common room	37 (26)	46 (32)	0.7 (0.4-1.2)	0.23
Engaged in group activities*	73 (51)	69 (48)	1.1 (0.7-1.9)	0.61
Attended 1 or more religious ceremonies*	6 (4)	15 (10)	0.3 (0.1-0.9)	0.04
Sports score 0	50 (35)	42 (29)	1.0	0.11
Sports score 1-17	25 (17)	23 (16)	0.9 (0.5-1.9)	0.83
Sports score 18-50	28 (19)	46 (32)	0.5 (0.2-0.9)	0.03
Sports score > 50	41 (29)	33 (23)	1.0 (0.5-2.0)	1.00

OR indicates Odds Ratio and CI indicates Confidence Interval

* in fortnight period before illness (cases) or interview (controls)

7.3.2.3 Other social behaviours

In the univariate matched analysis of other social variables there was no effect of socioeconomic status as measured by using the composite variable of car and home ownership, or by using the Registrar General's classification by occupation. Being below the control median for birth weight was not associated with being a case, but delivery at less than 37 completed weeks of gestation was. At the univariate stage of analysis, a history of being breast-fed as an infant was associated with protection against disease (see table 7).

Table 7. Univariate analyses for other social variables

Exposures	Cases n=144 (%)	Controls n=144 (%)	Matched OR (95%CI)	P value
Socio-economic status of head of household: Categorical variable based on home and car ownership (OR relative to owning neither home nor car)				
<i>No home and no car ownership</i>	12 (8)	12 (8)	1.0	-
<i>One or more cars but not own home</i>	22 (15)	16 (11)	1.4 (0.5-4.4)	0.5
<i>Own home but no car</i>	7 (5)	5 (4)	1.4 (0.3-6.6)	0.6
<i>One or more cars plus own home</i>	102 (71)	110 (76)	0.9 (0.4-2.3)	0.8
Subject's occupational status (OR relative to being employed)				
<i>Employed</i>	36 (25)	38 (27)	1.0	-
<i>University student</i>	50 (35)	46 (32)	1.2 (0.6-4.0)	0.7
<i>School student</i>	52 (36)	51 (36)	1.1 (0.4-2.8)	0.8
<i>Other</i>	5 (4)	8 (6)	0.6 (0.2-2.5)	0.5
Born at less than 37 completed weeks of pregnancy.	14 (10)	7 (5)	2.2 (0.8-5.7)	0.12
Breast fed as infant	98 (69)	101 (72)	0.8 (0.4-1.4)	0.12
Birth weight < 3.36kg (control median)	65 (48)	65 (48)	1.0 (0.6-1.6)	0.9

OR indicates Odds Ratio and CI indicates Confidence Interval

7.3.3 Psychological hypotheses

The main psychological hypotheses concerned the effect of life stress and social support networks on the risk of meningococcal disease in adolescents.

7.3.3.1 Life stress and social support networks

The A-file life stress scale consists of six individual scales relating to different elements of adolescent life stress (family changes, sexual matters, losses, responsibilities & strains, substance abuse, legal matters). Each scale has a stress measure and for the purposes of this study a distress measure was also added. A higher score indicates a higher perceived level of stress or distress. Mean scores for stress and distress for each scale along with mean scores for total stress and total distress were calculated. Results are presented in table eight.

Stress and distress relating to family responsibilities and strains, and stress and distress relating to losses within the family, over the year prior to admission, were all associated with protection against disease in univariate analysis. Measures of social support and/or of perceived satisfaction with the social support provided were not associated with disease or protection against disease.

Table 8. Univariate analysis for life stress and social support

Variable	Cases n=144	Controls n=144	Mean score (cases)	Mean score (controls)	MatchedOR (95% CI)	P value
Total stress	142	138	6.1	6.8	1.0 (0.9-1.0)	0.24
Total distress	142	138	24	28.2	1.0 (0.98-1.0)	0.2
Stress family changes	144	142	1.4	1.4	1.0 (0.8-1.2)	0.97
Distress family changes	144	142	3.3	3.2	1.0 (1.0-1.1)	0.75
Stress family sexual matters	143	144	0.3	0.3	0.8 (0.5-1.2)	0.21
Distress family sexual matters	143	144	0.7	0.9	1.0 (0.9-1.1)	0.38
Stress family losses	143	144	0.4	0.6	0.77 (0.57-1.05)	0.1
Distress family losses	143	144	2.2	4.2	0.96 (0.92-1.0)	0.03
Stress family responsibilities & strains	143	139	3.3	3.9	0.92 (0.84-1.01)	0.07
Distress family responsibilities & strains	143	139	17.1	22	0.99 (0.98-1.0)	0.06
Stress school & substance abuse	144	144	0.5	0.5	1.1 (0.81-4)	0.63
Distress school & substance abuse	144	144	2.0	2.0	1.0 (1.0-1.1)	0.96
Stress family legal matters	144	144	0.1	0.1	1.4 (0.7-3.0)	0.36
Distress family legal matters	144	144	0.7	0.5	1.0 (0.9-1.2)	0.48
Social support	143	144	3.09	3.09	1.0 (0.8-1.2)	0.96
Social satisfaction	143	143	5.29	5.41	0.8 (0.6-1.1)	0.21
Social support & social satisfaction	143	143	3.95	4.0	1.0 (0.7-1.2)	0.68

OR indicates Odds Ratio and CI indicates Confidence Interval
 All variables relate to the year prior to admission (cases) or interview (controls)

7.4 Multivariate analysis

Factors significant at a level of 0.2 or less during univariate analysis or those that formed a major hypothesis of this study (eg. MBL levels, student status) were entered into a multivariate logistic regression model along with socio-economic status, occupation and our seasonal variable.

In the final model, factors independently associated with higher risk of MD in adolescents were history of preceding illness, intimate kissing, being a student and preterm birth (see table 9). Factors independently associated with lower risk included religious observance and having received a vaccine against serogroup C meningococci. Multivariate analysis using only microbiologically confirmed cases resulted in less power but gave similar results except for preterm birth (OR = 2.3, 95% CI: 0.5-10.0, $p=0.3$) and attendance at religious ceremonies (OR = 0.13, 95% CI: 0.01-1.4, $p=0.09$). Passive smoking and use of alcohol and illegal drugs were not independently associated with disease. Bedroom sharing and the other social contact and leisure variables lost significance in the multivariate model. The stress and distress variables important during univariate analysis were not independently associated with protection in the multivariate model. Use of the Registrar General's classification for parental occupation as a measure of SES instead of the composite variable of car / home ownership, did not materially change the final model. No significant interactions were found.

Table 9. Multivariate analysis of risk factors for MD

Exposures	Matched OR (95% CI)	P value
Vaccinated against serogroup C Meningococcus	0.12 (0.04-0.37)	<0.001
Multiple intimate kissing contacts*	3.7 (1.7-8.1)	0.001
Attended 1 or more religious ceremonies*	0.10 (0.02-0.58)	0.01
Preceding illness*	2.9 (1.4-5.9)	0.003
Born at less than 37 completed weeks of pregnancy	3.7 (1.0-13.5)	0.05
Occupational status (OR relative to being employed)		
<i>University student</i>	3.4 (1.2-10.0)	0.03
<i>School student</i>	3.3 (0.8-13.4)	0.1
Season	5.6 (1.9-16.3)	0.001
Socio-economic status by head of household car and home ownership (relative to no home, no car)		
<i>No home, > 0 cars</i>	3.6 (0.8-17.2)	0.11
<i>Home, no car</i>	1.5 (0.2-13.0)	0.7
<i>Home, >0 cars</i>	1.6 (0.4-6.4)	0.49

OR indicates Odds Ratio and CI indicates Confidence Interval. OR adjusted for all other factors shown and controlled for parental car/home ownership and MD season. Data for all variables available in 130 pairs. Excluding the gestation variable did not confound OR estimates and model then included 136 pairs.

*in fortnight period before illness (cases) or interview (controls)

8 Discussion

8.1 Summary of findings

In this population-based, case-control study of risk factors for the development of meningococcal disease in adolescents aged 15 to 19 years, reporting a preceding illness in the fortnight prior to admission to hospital, having 2 or more intimate kissing contacts in this period, being a student and being born at less than 37 completed weeks of gestation were all independently associated with increased risk of disease. Having received meningococcal serogroup C vaccination and attending religious ceremonies at least once during the fortnight prior to admission were associated with protection against disease.

Although reported preceding illness was a strong risk factor, the specific respiratory viruses responsible could not be identified and in particular there was no association between influenza and the subsequent development of meningococcal disease. No link was demonstrated between low MBL levels and MD. Contrary to the original hypotheses, active smoking, engagement in elite athletics and high stress levels were not found to be associated with disease in adolescents.

8.2 Strengths of this study

To date this is the largest population based study of risk factors for MD in a general population of adolescents and the only specific epidemiological investigation of disease risk during the adolescent peak. Only one other study has investigated risk factors specifically in young people [41]. However, this study examined risk factors in American college students rather than in a general population of adolescents, and subjects were from an older age group. The adolescents in their study were between 18 and 23 years of age which is past the age band of peak incidence in the United Kingdom.

As carrying out this study involved travelling significant distances to visit adolescents and was performed at a time of relatively high incidence of disease, it is unlikely that this study could easily be replicated. Other developed countries such as Australia or the United States are geographically large and the incidence of this disease has decreased in many countries after the introduction of routine vaccination. This means that the results of this study provide a unique picture of the risk profile for meningococcal disease in an adolescent age group.

There are many different facets to the risk profile for meningococcal disease. This study has attempted to encompass many of these by adopting a biopsychosocial approach, focusing on elements prominent in the adolescent life phase. While many of the variables assessed have been examined in previous studies involving subjects of all ages, several novel variables more specific to adolescent behaviour have also been included. In addition, when addressing the issue of preceding illness as a risk factor for disease, both symptom-based and laboratory-based definitions for preceding illness were adopted in contrast to many other studies that employed either one or the other approach.

Although case-control studies have inherent problems (see section 8.4) they are able to provide important findings in a relatively short time, especially when the incidence of the outcome (in this case meningococcal disease) is low. This study could not have been approached in any other manner given the small numbers of potential study subjects.

8.3 Comparisons with previous work

8.3.1 Biological factors

The first area of focus in this investigation was that of biological risk factors for disease. The effect of preceding illness with respiratory viruses and the effect of low MBL levels on disease risk were examined.

8.3.1.1 Preceding illness

A history of preceding illness in the fortnight prior to the development of meningococcal disease was an independent biological risk factor. Preceding illness

has been identified as a risk factor in other studies [^{41;44;119}] and this was confirmed in this investigation using a similar symptom-based definition of preceding illness.

Other studies have failed to adjust for the effect of season in their analysis. As both meningococcal disease and respiratory viral infection, in particular influenza, are highly seasonal this could have implications for the results of such studies. A “time-of-year” effect was therefore included in our analysis in order to control for the potential seasonal difference between interviewing cases and controls [²⁴⁵]. The effect of preceding illness remained unchanged after adjustment for high season of MD or influenza.

A possible criticism of this symptom-based approach is that the preceding illness identified as a risk factor may in fact be just the prodrome of the ensuing meningococcal infection rather than a true preceding illness. This would clearly lead to an overestimation of the risk that preceding illness contributes. This study methodology attempted to clearly separate these two entities during data collection from subjects. During further data analysis (performed by Pietro Coen and therefore not presented in this thesis) two clearly distinct symptom profiles could be identified for preceding illness and prodromal illness. The preceding illness was characterised by sore throat, cough and runny nose and occurred at a median of 9 days prior to meningococcal illness, while the prodromal illness was characterised by fever, rigors and muscle aches and occurred at a median of 1 day prior to meningococcal illness. These two symptom profiles were statistically significantly different, suggesting that a preceding illness could confidently be identified and that the presence of a prodromal illness did not confound our preceding illness result.

The precise aetiology of the preceding illness is unclear but is probably a heterogeneous array of respiratory viruses. In this study an attempt was made to confirm this aetiology using a laboratory-based approach alongside our symptom-based definition. However, we found no association between influenza, RSV, mycoplasma or chlamydia viruses and meningococcal disease. This is in contrast to some published work, especially with regards to influenza. Outbreak [²⁹⁻³¹] and surveillance data linkage studies [³³] suggest influenza predisposes to MD, while a case-control study in sub-saharan Africa implicated adenovirus, parainfluenza, rhinovirus, mycoplasma and RSV in the aetiology of serogroup A meningococcal

meningitis [32]. Interestingly this study also did not isolate influenza viruses. However, a recent case-control study from Australia also failed to demonstrate a role for influenza [119] and Stuart et al [38] found no epidemiological evidence for RSV being a predisposing agent.

The role of EBV in the aetiology of meningococcal disease in teenagers remains intriguing. In this study no statistical association between EBV infection and MD was found although the only three IgM positive results occurred in cases, and cases were also more likely to be IgG positive ($p=0.12$ in univariate analysis). To our knowledge there has been no previous work investigating this link and further exploration of this risk factor could be justified.

It seems that while the symptom-based results confirm previously published associations between preceding illness and meningococcal disease, the laboratory-based results generally contrast with previous work in that a link between meningococcal infection and respiratory viruses, in particular influenza, was not demonstrated. There are several possible explanations for this;

Firstly that there is no actual link and that previous epidemiological and linkage studies were flawed. However, although initial observational work investigating the link between influenza and MD was based on very small numbers [28-30], later studies were larger. Watson used “deseasonalised” data in an ecological study [33] to support his suggestion that influenza infection was associated with meningococcal disease, although he concluded that the risk attributable to influenza was likely to be very low. Cartwright found cases to be nearly four times as likely as controls to demonstrate serological evidence of recent influenza infection [31].

Secondly, the largest laboratory-based study [32] was performed using subjects from a developing country and it is conceivable that the wide array of viruses they identified was due to the much higher proportion of patients carrying these viruses than would be found in a developed country. It is interesting that this study was unable to identify influenza viruses amongst the viruses isolated.

Thirdly, it is possible that in this study there was inadequate power to detect an effect that was present. However, the PCR results (influenza, chlamydia, mycoplasma, RSV) did not produce a single positive result and numbers were larger than those

included in the African cohort. It is important to emphasise that the subjects in this study may no longer have been shedding virus by the time of recruitment (a large geographical area meant specimen collection might be delayed). Samples were frequently not collected until the 3rd or 4th day of admission to hospital. In addition to PCR, serological techniques were also employed in an attempt to clarify this issue. For 81 case subjects paired serology was obtained (acute and convalescent sera) and in 11% and 6% respectively a four-fold rise for influenza A and B was demonstrated. Using cut-off titres to compare case and control sera and controlling for season of sampling, serological results for influenza failed to establish any link with case status. Approximately 15% of teenagers contract influenza each year (personal communication Dr Maria Zambon), which is consistent with the 17% seroconversion rate in this study. This is additionally suggestive of a problem with our PCR sampling, rather than a lack of power or association.

Finally, our specimen collection and storage methods might have been inadequate, despite following rigorous guidelines from the Public Health Laboratory Services Influenza Laboratory. Although we accept that this could apply to our PCR samples, we still obtained meaningful results for serological tests and these remained inconclusive.

8.3.1.2 Mannose-binding lectin

To our knowledge this is the only study examining the relationship between MBL production and meningococcal disease in a population of adolescents. No significant relationship between genetically determined low levels of MBL production and disease risk was demonstrated, using information concerning both structural gene mutations and promoter region polymorphisms. This is in contrast to evidence that MBL deficiency is a risk factor in early childhood [62,68]. Although a failure to find an association between low MBL production and disease might be due to lack of power, these results do support recent suggestions that MBL may become less important in protecting against MD with increasing age as elements of the acquired immune system mature [68-70]. While it is likely that these defects in the innate immune system have a major role to play in childhood susceptibility to MD, other mechanisms including environmental factors may become more important in the susceptibility profile of teenagers and young adults.

8.3.1.3 Other biological factors

At the univariate stage of analysis, antibiotic usage in the fortnight prior to admission (cases) or interview (controls) was significantly protective against the development of disease, despite relatively small numbers. This effect disappeared after insertion in a multivariate model that included preceding illness and socio-economic status. It is conceivable that control subjects, who were of slightly higher socio-economic status (see table 2), access health care to a greater or more effective extent than cases, thereby receiving antibiotic treatment for their preceding illness more frequently than might the case subjects.

Previous vaccination against serogroup C meningococcal infection was significantly protective against the development of disease.

8.3.2 Social factors

The second area of investigation was the effect of social behavioural factors on adolescent risk of disease. These included substance use behaviours, in particular the role of active and passive smoke exposure, social contact behaviours and leisure pursuits.

8.3.2.1 Substance use

There was no significant role for active or passive smoke/smoker exposure in adolescents with meningococcal disease. The result for active smoke exposure is generally consistent with other studies examining the effect of active smoking in young adults [41;44;249]. The lack of association of passive smoking with MD is in contrast with evidence that passive smoke exposure is a risk factor in children [43;44;74;75;119]. However several other studies have also been unable to confirm these findings in an adolescent or adult population [41;43]. It seems likely that while young children are susceptible to the effects of passive smoke exposure, especially within the home environment, adolescents display a lesser risk of disease from this exposure. Children exposed to passive smoke are more likely to be exposed to meningococci because of higher carriage rates in their smoking parents [76]. They tend to have close and intimate contact with their parents and are therefore at increased risk of transmission through kissing or coughing. It might be postulated that these children become transient asymptomatic carriers (provided they don't

develop disease) and therefore develop protective immunity early in life. It is possible that they therefore become less vulnerable to the effects of smoke exposure as older teenagers. In addition most studies emphasise the role of either maternal passive smoke exposure [74;75] or smoke exposure within the home environment [43;44;85] in the risk profile of young children. In addition to the passive smoke exposure variables measured, as adolescents are likely to spend less time with their mothers and within the home than are younger children, this may also contribute to the reduced effect of passive smoke exposure in teenagers seen in this study. As active smoking does not appear to predispose to meningococcal disease, it could be suggested that it is exposure to smokers with higher carriage rates that confers any increased risk of passive smoke exposure, rather than a direct biological effect of smoke on nasopharyngeal defences and humoral immunity (see appendix 28).

In this study there was no independent effect of alcohol consumption on the risk of meningococcal disease in teenagers. This result confirms the findings of Bruce et al in their population of young adults [41]. Both this study and the study by Bruce et al found alcohol consumption to be a risk factor at univariate analysis suggesting that this behaviour reflects social mixing and other high-risk behaviours associated with drinking rather than alcohol consumption per se. It is possible that alcohol lies on the causal pathway to disease, making a small contribution by altering social behaviours that increase risk. The single study reporting a true association was almost certainly reflecting this [98].

Similarly the use of illicit drugs played no role in the aetiology of meningococcal disease in teenagers. This is a novel finding as there are no previous studies examining this issue. The regular consumption of drugs and the consumption of drugs in the fortnight prior to admission or interview were significant risk factors at the univariate stage of analysis. However this effect disappeared during multivariate analysis suggesting it to be a marker for other high-risk behaviours and contacts in the same way that alcohol use appears to be. It is also possible that too few subjects reported drug-related behaviours and that larger studies are needed to show any effect.

8.3.2.2 Social contact & leisure behaviours

A number of adolescent health behaviours were significantly associated with the development of meningococcal disease at univariate level of analysis (sharing a bedroom, attending pubs or bars, attending a nightclub, disco or party, and visiting friends' houses daily). These variables reflect social mixing associated with other behaviours that are likely to increase risk, and that may lie on a causal pathway (crowded conditions, close proximity of communication due to high background noise levels, alcohol, passive smoke exposure). However only deep (intimate) kissing with multiple partners remained independently significant in the multivariate model. This association has not been noted before in studies of college students and older adults [41;119]. Kissing on the mouth has been suggested as a risk factor in children [43] but there was no evidence supporting this in adolescents. Intimate kissing has been reported as a risk factor for carriage of meningococci in university students [250] and it is likely that intimate kissing with multiple partners increases risk of transmission. Sharing a bed/bedroom and having a regular partner were not significant in the model if intimate kissing was included, suggesting that risk derives from oropharyngeal exchange with more than one person rather than other behaviours related to proximity.

Both university and school students were at increased risk of MD compared with those in employment. This is in agreement with another major UK study [117] but not with research from the United States [118]. Student status may increase risk through crowded campus living and increased social mixing, or through increased "risky" behaviours compared with employed young people of the same age. Contrary to other studies [41;118] living in dormitory-style accommodation did not increase risk, however numbers were very small (seven percent of cases, six percent of controls). It is interesting to note that there was no significant association between student status and disease at univariate analysis ($p=0.7$), but a significant association emerged when this was entered into the multivariate model ($p=0.03$). Part of the motivation for the design of this study was the singular conception amongst the public and parts of the medical profession that the most important exposure conferring risk was that of being a university student, especially if residing in dormitory accommodation. This clearly forms only part of the risk profile in this age group.

There was no relationship between crowded living conditions and meningococcal disease which is contrary to the cardinal early work in the epidemiology of meningococcal disease [108], and with more recent studies [43;113]. However, it has been suggested that overcrowding needs to be extreme to influence disease and that when separated from social class and poverty it probably has little influence on health today [109]. It is likely that in our study and others that failed to find this association [41;75], overcrowding was not extreme enough to confer increased risk.

8.3.2.3 Exercise

To our knowledge this is the first study to examine a possible role for extreme exercise in the aetiology of meningococcal disease in teenagers. In the univariate analysis there was a protective effect of moderate amounts of exercise on risk of disease. This is in agreement with published work [71]. However in the multivariate model this association disappeared and there was no link between exercise and the development of disease in this age group. This is likely to be because of a correlation between moderate exercise and other behaviours that increase risk, for example student status. There was no suggestion of the J-shaped relationship proposed by Hughes [71] consisting of a decreasing risk of infection from mild through to moderate exercising and an increased risk with prolonged strenuous exercise.

Theories suggest that temporary immunosuppression resulting from extreme exercise might leave the host susceptible to a “window of opportunity” for infection. As clinical meningococcal disease is a relatively rare event, the number of cases occurring as a result of this temporary immunosuppression are likely to be extremely low. In addition, it is probable that the numbers of adolescents in our study exercising at a level sufficient to produce such immunosuppression was also very low.

8.3.2.4 Others

The association of preterm birth and meningococcal disease demonstrated in this study has not been previously described for adolescents. Prematurity has been associated with an increased risk of both infectious disease hospitalisation and mortality during early childhood [135;136], as well as with an increased risk of invasive meningococcal disease during the first year of life only [138]. Prenatal undernutrition

(as opposed to prematurity) has been shown to reduce antibody responses to vaccination in adolescents [¹³⁷], tentatively indicating that any effect of prenatal environment might persist. The association between premature delivery and meningococcal disease shown in this study may be a chance finding or could reflect real differences in immune function programming related to timing of birth, persisting beyond childhood. It is also conceivable that pre- and postnatal maternal lifestyle factors that make premature birth more likely, might also increase the risk of meningococcal disease in offspring, and that these factors were in operation in this study despite careful controlling for possible confounders. A history of premature delivery could be unreliable and it is therefore possible that this data has questionable validity given the retrospective study design and recall bias. The statistical significance of this result was lost when only microbiologically confirmed cases were included, but this is likely to be an effect of loss of power.

Although a history of breast-feeding in infancy was significantly associated with protection against development of disease during univariate analysis this effect disappeared after insertion in a multivariate model including socioeconomic variables. This replicates the negative findings from other studies examining risk factors in children [^{43;75;84}]. A single study from South Africa did however identify a history of breast-feeding for less than 3 months as being a risk factor for disease [⁴²]. It is unclear though whether this study adequately controlled for social class. While it is probable that breast-feeding could be protective against meningococcal disease in infancy [¹¹⁰], it is unlikely that this protective effect is prolonged into adolescence.

Recent religious attendance was linked to lower risk of MD, as has been reported elsewhere [⁷⁵]. The association was confirmed when the analysis was repeated for habitual religious attendance in the previous year (personal communication Jennie Borg), indicating minimal bias due to non-attendance of cases suffering preceding or prodromal illness. Religious observance has been associated with lower risk for all-cause mortality [¹³⁹], substance abuse and sexual risk-taking in adolescents [²⁵¹] and has beneficial immune effects [¹⁴⁰]. Health benefits might arise from the comforting belief that a spiritual world exists [²⁵²] or from the social support gained from a close community with shared beliefs. However, in our subjects perhaps the most plausible explanation for our finding is that religious attendance is associated with lifestyle

factors that promote health and protect against infection [¹³⁹] but that were not fully accounted for in multivariate analysis.

Investigation of the relationship between socio-economic status and disease was not a hypothesis of this study. Socio-economic status was not associated with disease at univariate or multivariate levels of analysis. This is in contrast to published work [^{43;74;75;85;128}] but is due to close matching for social class in this study cohort. It was necessary to closely match for this variable as it is a known risk factor for disease and the aim of this work was to identify other risk or protective factors operating.

8.3.3 Psychological factors

The final area of study involved the influence of stressful life events in the year prior to illness on risk of disease and evaluating the possible buffering effect of social support networks on this risk profile.

8.3.3.1 Stress

This is the only study examining the effect of stress on meningococcal disease risk during adolescence using a stress scale designed specifically for this age group.

Seven elements of daily life stress were studied. For each of these elements both the stress caused and the distress experienced were measured. During univariate analysis two of these elements, stress and distress over family losses and stress and distress over family responsibilities and strains, were associated with protection against meningococcal disease. However, when entered into a multivariate model, these elements of adolescent stress were no longer significant, leading to the conclusion that life stress is probably not linked to the development of meningococcal disease in the teenage population.

This result is generally consistent with another major UK study that found no evidence for an association between meningococcal disease and a total life events score [⁴³], although individual elements of this score were significantly associated with disease. A recent Australian study similarly failed to find any association between stressful life events and meningococcal disease [⁸⁴]. A study that did find exposure to stressful events to be associated with meningococcal disease [⁴⁴] used a vastly different scale for assessing stress in a mixed age group population.

Although the body of literature reviewed in section 5.1 suggests that stress might be a potential causal cofactor in the aetiology of infectious disease, this is by no means certain. There is little prior evidence that stress is important in the aetiology of meningococcal disease and this study has also been unable to establish a link, despite using a measurement scale that was simple and specific to teenagers. Adolescents as a group remain vulnerable to the effects of stress [^{157;158}], especially effects mediated through a behavioural pathway. However its role in the aetiology of this relatively uncommon infectious disease is questionable.

8.3.3.2 Social support

There was no evidence for the buffering effect of social support networks on life stress in adolescents. In addition the absence of social support networks did not act as an additional stressor thereby increasing the risk of developing meningococcal disease.

Although there is no previous published work examining the effect of social support networks on the risk of meningococcal disease, Rowlison found no support for the hypothesis that social support networks had any buffering effect on major life events per se within an adolescent population [²⁰⁵].

The assessment of social support is complex, especially within an adolescent population in whom social structures are undergoing many changes. The decline of family support alongside increased family conflict, coupled with the expansion of peer group support makes quantifying and qualifying social support networks difficult. Alongside its complicated relationship with life stress it is perhaps not surprising that no aetiological relationship between social support networks and MD was demonstrated.

8.4 Limitations of this study

When interpreting the results of case control studies, the effects of two phenomena must be considered. These are those of bias and confounding.

These study findings are susceptible to the biases common to case-control studies. Although such a study design can yield important findings within a relatively short time frame, it is more susceptible to bias than other comparative study designs, in

particular randomised trials [²⁵³]. A case-control design was the only possible manner in which this study could be conducted, so it is therefore important to remember the criteria set out by Schulz for conducting a good case-control study and make rigorous efforts to fulfill these, acknowledging potential sources of bias;

- **Clearly define criteria for being a case & eligibility criteria for selection**

The case selection criteria were clearly stated (see section 6.4.1). Although ideal, it was not practical to recruit only microbiologically confirmed cases. However, restricting analysis to subjects with microbiological confirmation showed minimal confounding of the identified risk factors, apart from preterm birth, which lost statistical significance once smaller numbers were analysed.

For ethical and practical reasons we excluded the small number of cases who died. This meant that our sample was biased towards less severe cases, and would, if anything, underestimate the effect of risk factor variables, especially those with a dose-response effect.

Case recruitment was population-based and prospective. However our overall recruitment rate from the incident cases occurring during the study period was relatively low at 48%. Even when only the referred case are considered, our recruitment rate still reached only 63%, although for the majority of these there is a clear reason for non-recruitment (see section 7.1.1.1). With low recruitment rates in case control studies selection bias can become a concern. In this study however, only 4.5% of eligible (referred) cases actually refused participation. A further 3% of referred cases (4.5% of recruited cases) later refused participation or were lost to follow-up. It is these two groups who may have characteristics systematically different from the selected group. It is unlikely that the remaining non-recruited cases were systematically different from recruited cases as cases were recruited from six out of a total of eight English health regions giving us a nationally representative sample. In addition, as referral to the study depended more on characteristics of the refereee and less on characteristics of the case, and as the other reasons for non-recruitment were randomly distributed, selection bias of cases was minimised as far as possible. However, a degree of bias cannot be excluded as the characteristics of non-recruited cases could not be investigated.

- **Controls should come from the same population as the cases & selection be independent of exposure of interest**

Selection of a control group is probably the most important methodological hurdle when undertaking a case control study [²⁵³]. The control group was population-based and selected largely at random from the general population in the same geographical area as the case. While 63% of our recruited controls were either the first or second approached to participate, 20% were the fourth or greater. This is a potential source of selection bias, as the characteristics of controls willing to participate in this kind of study may be different from those not willing to participate and may relate to risk of exposure.

Although the aim was to closely match cases and controls for socio-economic status, slightly more controls than cases came from higher social class groups as measured by car/home ownership and Registrar General's classification (see table 2). This has to be acknowledged as a potential source of bias.

- **Investigators should be blind to case-control status or to main hypotheses**

Where data collectors are not blinded to case control status information bias can result. Unfortunately it was not possible to blind data collectors to status. Acknowledging this, training was undertaken by data collectors to elicit information in a similar manner from cases and controls.

- **Elicit exposures in same manner from cases & controls & use memory aides to balance recall between cases and controls**

Recall bias can be a major problem in this type of study and the findings presented here may be subject to this type of bias. Efforts were made to avoid recall bias by firstly, using a short recall period. Some risk factor studies have chosen longer periods of time over which to question subjects about exposures [^{41;42;75}], but in keeping with another major UK study [⁴³] and considering the age group of our subjects, a fortnight period was considered to be more appropriate.

Secondly, memory aides were employed to assist subjects' responses. Adolescents often keep a diary and where this was the case, we asked the subject to refer to it

during the interview. Diaries kept for school or college were often also employed. Timelines were produced at each interview in an effort to recreate the fortnight period under question and therefore stimulate memory.

Finally a different recall period was used for cases and controls. Other studies have questioned controls about the period of time directly preceding the case admission [41:44]. This was felt to be too challenging for our group of adolescents and would have introduced a greater degree of recall bias than the bias introduced through interviewing and sampling in different seasons.

- **Address confounding with careful study design or during analysis**

Potential confounding variables should be carefully identified so that data can be obtained and used to control for such confounders during analysis. An example of this is the creation of a seasonal variable to control for the confounding effect of interviewing and taking blood samples from cases and controls during different seasons (see section 7.3.1.1). A further potential source of bias arises from preceding or prodromal illness, as a reduction in risk behaviours in those who are becoming ill may underestimate the effect of risk factors for MD and over-estimate protective effects. However, analysis of long-term habitual data on active smoking and religious attendance indicated that behaviour change due to illness was not a significant source of confounding bias (personal communication Jennie Borg).

Although every effort was made during design and analysis to reduce the effect of confounding, some residual confounding will invariably remain and be responsible, at least in part, for some of the results obtained in this study.

While discussing limitations of this study and bias, it is important to mention social desirability bias. This type of bias arises when subjects report exposures in a manner that they perceive will be acceptable to the interviewer. This is of particular importance in this study as information about socially sensitive behaviours (smoking, alcohol use, substance abuse, intimate relationships) was being requested from a group of adolescents. It is unlikely that our results were significantly affected by this type of bias as they are comparable with nationally reported statistics on the prevalence of such behaviours [93:106].

As previously mentioned confounding is an important consideration when interpreting the results of a study such as this. Confounding can be conceptualised as a mixing of the effect of the exposure under study on the disease with that of a third factor. This third factor must be associated with the exposure and, independent of that exposure, be a risk factor for disease. Confounding can therefore lead to an over- or underestimate of the true association between the exposure under study and the disease [²⁵⁴]. It is important to collect data on as many confounding variables as possible in order that they can be considered during analysis.

There are a number of methods that can be employed, both in study design and analysis, to control for confounding. In this study matching was employed to control for background confounders (sex, age, SES), and multivariate conditional logistic regression analysis was employed at the analytical phase to take into account all possible confounding variables on which data were collected. However it must be remembered that residual confounding (confounding that has not been accounted for during design or analysis) can still operate and be partly or wholly responsible for results seen.

A further possible limitation of this study concerns case recruitment and sample collection. Personnel other than study investigators recruited case subjects into this study. In addition, the samples taken from case subjects were taken by local hospital personnel and not by the study investigators. Controls were largely recruited into the study and samples taken by study personnel. This may have had an effect on differential recruitment and sampling rates between cases and controls.

In summary, the chief limitations of this study were a relatively low recruitment rate for cases, and that one-fifth of controls were not in our first selected three. In addition, there will be a degree of recall bias, unavoidable in case control studies but exaggerated by the delay between identification of subjects and interview. Many of the difficulties encountered were as a result of including a wide geographical area. This was necessary to achieve adequate statistical power as meningococcal disease is an uncommon disease. However, if repeating the study many of these problems could be overcome by appointing dedicated investigators in regional centres. This would obviously entail greater collaboration and inevitably more expense.

8.5 Conclusions

Epidemiology is constantly being refined by attempts to get closer to defining which exposure or what exposures actually increase the risk of a given disease. This study represents a detailed attempt to define the psychosocial geography of teenage activity in cases and closely matched controls, giving real hope of achieving this. However, it is likely that while a number of independent risk factors were identified in this study there is in fact a multiplicity of risk factors operating in each individual in the final causal pathway to disease. Many of the risk factors found to be important at univariate analysis, no longer assumed significance once entered into a multivariate model. These factors may well still form part of the risk profile by making small contributions to a complex causal pathway.

This is the first study to examine risk factors for the development of meningococcal disease specifically within an adolescent population. The results provide a clearer picture of the risk profile for meningococcal disease in teenagers and how this compares with the risk profile in children. The pattern of risk and protection identified for MD in adolescence was different from that seen in younger children. Intimate kissing with multiple partners, preceding illness, being a student and preterm birth conferred higher risk of disease, whereas religious attendance and receipt of a meningococcal vaccine were associated with lower risk. Factors that are important in MD risk in younger children, such as passive smoking and deficiency of MBL, were not significant in adolescence.

The results of this investigation leave a few unanswered questions. The findings relating to EBV and meningococcal disease were interesting, and considering a plausible physiological basis for a link exists, further investigation of the role of EBV infection in meningococcal disease would be warranted. However, the logistic difficulties of further work in this area would have to be acknowledged. Potential for further investigation into the possible links between meningococcal disease in this age group and premature delivery also exists. Although the possibility of the finding relating prematurity to MD being a statistical nuance is acknowledged, greater numbers may well yield more meaningful results.

The findings indicate that personal behaviours may moderate the risk of MD in adolescence. Targeted health promotion campaigns aimed at emphasising the risk of multiple deep kissing contacts, for example during introductory weeks at university or college (“freshers week”), might have limited effect. Similarly greater awareness of the need to “look out for your mates” during periods of influenza-like illnesses or hangover days, might prevent the occasional case of mis-diagnosed meningococcal disease occurring in a student alone in their bedroom. However it is unlikely that behaviour-based health promotion messages for teenagers will have significant impact in reducing risk of disease. The development of further effective meningococcal vaccines remains a key public health priority [²⁵⁵] in the fight against this rare but devastating disease.

Reference List

1. Booy R, Habibi P, Nadel S, deMunter C, Morrison A, Levin M *et al.* Reduction in case fatality rate from meningococcal disease associated with improved healthcare delivery. *Arch.Dis.Child* 2001;**85**:386-90.
2. Anderson MS, Glode MP, Smith AL. Meningococcal disease. In Feigin, Cherry, eds. *Textbook of Paediatric Infectious Diseases*, pp 1143-56. W.B.Saunders Company, 1998.
3. Pathan N, Faust SN, Levin M. Pathophysiology of meningococcal meningitis and septicaemia. *Arch.Dis.Child* 2003;**88**:601-7.
4. Welch SB, Nadel S. Treatment of meningococcal infection. *Arch.Dis.Child* 2003;**88**:608-14.
5. Thorburn K, Baines P, Thomson A, Hart CA. Mortality in severe meningococcal disease. *Arch.Dis.Child* 2001;**85**:382-5.
6. Public Health Laboratory Service, Public Health Medicine Environmental Group, Scottish Centre for Infection and Environmental Health. Guidelines for the public health management of meningococcal disease in the UK. *CDR Weekly* 2002;**5**:187-204.
7. Strang JR, Pugh EJ. Meningococcal infections: reducing the case fatality rate by giving penicillin before admission to hospital. *BMJ* 1992;**305**:141-3.
8. Cochrane Injuries Group Albumin Reviewers. Human albumin administration in critically ill patients; systematic review of randomised controlled trials. *BMJ* 1998;**317**:235-40.
9. Balmer P, Miller E. Meningococcal disease: how to prevent and how to manage. *Current opinion in Infectious Diseases* 2002;**15**:275-81.
10. Judge D, Nadel S, Vergnaud S, Garralda ME. Psychiatric adjustment following meningococcal disease treated on a PICU. *Intensive Care Medicine* 2002;**28**:648-50.
11. Lystad A, Aasen S. The epidemiology of meningococcal disease in Norway 1975-1991. *NIPH Annals* 1991;**14**:57-66.

12. Stuart JM, Cartwright K, Dawson J, Rickard J, Noah ND. Risk factors for meningococcal disease: a case control study in south west england. *Community Medicine* 1988;**10**:139-46.
13. Connolly M, Noah ND. Is group C meningococcal disease increasing in Europe? A report of surveillance of meningococcal infection in Europe 1993-6. *Epidem.Inf.* 1999;**122**:41-9.
14. Ramsay, M and Kaczmarski, E. B. The epidemiology of meningococcal disease in England and Wales, 1997/8. 1999.
15. Coen PG, Cartwright K, Stuart JM. Mathematical modelling of infection and disease due to *Neisseria meningitidis* and *Neisseria lactamica*. *Int J Epidemiol* 2000;**29**:180-8.
16. Cartwright KAV, Stuart JM, Jones D, Noah ND. The Stonehouse survey: nasopharyngeal carriage of meningococci and *Neisseria lactamica*. *Epidem.Inf.* 1987;**99**:591-601.
17. Gold R, Goldschneider I, Lepow ML, Draper TF, Randolph M. Carriage of *Neisseria meningitidis* and *neisseria lactamica* in infants and children. *Journal of Infectious Diseases* 1978;**137**:112-21.
18. Jones D, Mallard RH. Age incidence of meningococcal infection England and Wales, 1984-1991. *J.Infect.* 1993;**27**:83-8.
19. Borrow R, Fox AJ, Cartwright K, Begg N, Jones DM. Salivary antibodies following parental immunisation of infants with a meningococcal serogroup A and C conjugated vaccine. *Epidem.Inf.* 1999;**123**:201-8.
20. Shigematsu M, Davison KL, Charlett A, Crowcroft NS. National enhanced surveillance of meningococcal disease in England, Wales and Northern Ireland, January 1999 - June 2001. *Epidem.Inf.* 2002;**129**:459-70.
21. Davison KL, Ramsay M, Crowcroft NS, Lieftucht A, Kaczmarski EB. Estimating the burden of serogroup C meningococcal disease in England and Wales. *Communicable Disease Public Health* 2002;**5**:213-9.
22. El Bashir H, Harding D, Booy R. Meningococcal serogroup C disease in England and Wales, 1993-2002. *in press* 2005.
23. Jones DM. Meningococcal vaccines. *Journal of medical microbiology* 1993;**38**:77-8.
24. Donaldson, L, Moores, Y, and Howe, J. Introduction of immunisation against group C meningococcal disease. 1-6. 20-7-1999. Department of Health.

25. Trotter CL, Ramsay M, Kaczmarski EB. Meningococcal serogroup C conjugate vaccination in England and Wales: coverage and initial impact of the campaign. *Communicable Disease Public Health* 2002;**5**:220-5.
26. von Pirquet C. Das Verhalten der kutanen Tuberkulin-reaktion wahrend der Masern. *Dtsch Med Wochenschr* 1908;**34**:1297-300.
27. Dagan R, Hall CB, Menegus MA. Atypical Bacterial Infections Explained by a Concomitant Virus Infection. *Pediatrics* 1985;**76**:411-4.
28. Lal HB, Naravan IK, Kalta SI. Simultaneous outbreaks of influenza and meningitis. *Journal Indian Medical Association* 1963;**40**:113-5.
29. Young LS, LaForce FM, Head JJ, Feeley JC, Bennett JV. A simultaneous outbreak of meningococcal and influenza infections. *N.Engl.J.Med.* 1972;**287**:5-9.
30. Harrison LH, Armstrong CW, Jenkins SR, Harmon MW, Ajello CW, Miller GB *et al.* A Cluster of Meningococcal Disease on a School Bus Following Epidemic Influenza. *Arch Intern Med* 1991;**151**:1005-9.
31. Cartwright K, Jones D, Smith AJ, Stuart JM, Kaczmarski EB, Palmer SR. Influenza A and meningococcal disease. *The Lancet* 1991;**338**:554-7.
32. Moore PS, Hierholzer J, DeWitt W, Gouan K, Djore D, Lippeveld T *et al.* Respiratory Viruses and Mycoplasma as Cofactors for Epidemic Group A Meningococcal Meningitis. *Journal American Medical Association* 1990;**264**:1271-5.
33. Watson JM, Nicholas S, James P. Meningococcal infection and influenza: do surveillance data support an association? In Brown LE, Hampson AW, Webster RG, eds. *Options for the control of influenza*, pp 82-4. London: Elsevier Science, 1996.
34. Kleemola M, Kayhty H. Increase in Titres of Antibodies to *Mycoplasma pneumoniae* in Patients with Purulent Meningitis. *J.Inf.Diseases* 1982;**146**:284-7.
35. Krasinski K, Nelson JD, Butler S, Luby JP, Kusmiesz H. Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. *American Journal of Epidemiology* 1987;**125**:499-507.
36. Abramson JS. The pathogenesis of bacterial infections in infants and children: The role of viruses. *Perspectives in Biology and Medicine* 1988;**32**:63-72.

37. Raza MW, Ogilvie MM, Blackwell CC, Stewart J, Elton RA, Weir DM. Effect of respiratory syncytial virus infection on binding of *Neisseria meningitidis* and *Haemophilus influenzae* type b to a human epithelial cell line (HEp-2). *Epidem.Inf.* 1993;**110**:339-47.
38. Stuart JM, Cartwright K, Andrews NJ. Respiratory syncytial virus infection and meningococcal disease. *Epidem.Inf.* 1996;**117**:107-11.
39. Andiman WA. The Epstein-Barr virus and EB virus infections in childhood. *The Journal of Pediatrics* 1979;**95**:171-82.
40. Tischendorf P. Development and persistence of immunity to EB virus in man. *Journal of Infectious Diseases* 1970;**122**:401.
41. Bruce MG, Rosenstein NE, Capparella JM, Shutt KA, Perkins BA, Collins M. Risk Factors for Meningococcal Disease in College Students. *Journal American Medical Association* 2001;**286**:688-93.
42. Moodley JR, Coetzee N, Hussey G. Risk factors for meningococcal disease in Cape Town. *South African Medical Journal* 1999;**89**:57-9.
43. Stanwell-Smith RE, Stuart JM, Hughes AO, Robinson P, Griffin MB, Cartwright K. Smoking, the environment and meningococcal disease: a case control study. *Epidem.Inf.* 1994;**112**:315-28.
44. Haneberg B, Tonjum T, Rodahl K, Gedde-Dahl TW. Factors preceding the onset of meningococcal disease, with special emphasis on passive smoking, stressful events, physical fitness and general symptoms of ill health. *NIPH Annals* 1983;**6**:169-73.
45. Tyrell DAJ, Cohen S, Schlarb JE. Signs and symptoms in common colds. *Epidem.Inf.* 1993;**111**:143-56.
46. Stephens DS, Hoffman LH, McGee ZA. Interaction of *Neisseria meningitidis* with Human Nasopharyngeal Mucosa: Attachment and Entry into Columnar Epithelial Cells. *J.Inf.Diseases* 1983;**148**:369-75.
47. Rouse BT, Horohov DW. Immunosuppression in viral infections. *Rev Infect Dis* 1986;**8**:850-67.
48. Junker AK, Ochs HD, Clark EA, Puterman ML, Wedgwood RJ. Transient Immune Deficiency in Patients with Acute Epstein-Barr Virus Infection. *Clinical Immunology and Immunopathology* 1986;**40**:436-46.

49. Skoner DP, Whiteside TL, Wilson JW, Doyle WJ, Herberman RB, Fireman P. Effect of Influenza A Virus Infection on Natural and Adaptive Cellular Immunity. *Clinical Immunology and Immunopathology* 1996;**79**:294-302.
50. Abramson JS, Mills EL. Depression of Neutrophil Function Induced by Viruses and its Role in Secondary Microbial Infections. *Rev Infect Dis* 1988;**10**:326-41.
51. Kim PE, Musher DM, Glezen WP, Rodriguez-Barradas MC, Nahm WK, Wright CE. Association of Invasive Pneumococcal disease with Season, Atmospheric Conditions, Air Pollution, and the Isolation of Respiratory Viruses. *Clin. Infect. Dis* 1996;**22**:100-6.
52. Larson HE, Blades R. Impairment of human polymorphonuclear leucocyte function by influenza virus. *The Lancet* 1976;**1**:283.
53. Martin RR, Couch RB, Greenberg SB, Cate TR, Warr GA. Effects of Infection with Influenza Virus on the Function of Polymorphonuclear Leukocytes. *J. Inf. Diseases* 1981;**144**:279.
54. Laurichesse H, Dedman D, Watson JM, Zambon M. Epidemiological features of parainfluenza virus infections: Laboratory surveillance in England and Wales, 1975-1997. *European Journal of Epidemiology* 1999;**15**:475-84.
55. Chang RS, Char DFB, Jones JH, Halstead SB. Incidence of Infectious Mononucleosis at the Universities of California and Hawaii. *J. Inf. Diseases* 1979;**140**:479-86.
56. Evans AS, Niederman JC. Epstein- Barr Virus. In Evans AS, ed. *Viral Infections of Humans: Epidemiology and Control*, pp 253-80. 1998.
57. Jack DL, Dodds AW, Anwar N, Ison CA, Law A, Frosch M *et al.* Activation of complement by mannose-binding lectin on isogenic mutants of *Neisseria meningitidis* Serogroup B. *The Journal of Immunology* 1998;1346-53.
58. Jack DL, Jarvis GA, Booth CL, Turner MW, Klein NJ. Mannose-Binding Lectin Accelerates Complement Activation and Increases Serum Killing of *Neisseria meningitidis*. *J. Inf. Diseases* 2001;**184**:836-45.
59. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. *The Lancet* 1989;1236-9.
60. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding Lectin Binds to a Range of Clinically relevant Microorganisms and promotes Complement Deposition. *Infection and immunity* 2000;**68**:688-93.

61. Neth O, Hann I, Turner MW, Klein NJ. deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. *The Lancet* 2001;**358**:614-8.
62. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. *BMJ* 1997;**314**:1229-31.
63. Hartshorn KL, Sastry K, White MR, Anders EM, Super M, Ezekowitz RA *et al*. Human Mannose-binding Protein Functions as an Opsonin for Influenza A Viruses. *J.Clin.Invest.* 1993;**91**:1414-20.
64. Van Emmerik LC, Kuijper EJ, Fijen CAP, Dankert J, Thiel S. Binding of mannan-binding protein to various bacterial pathogens of meningitis. *Clinical and experimental immunology* 1994;**97**:411-6.
65. Sumiya M, Summerfield JA. Mannose-binding protein, genetic variants and the risk of infection. *Q J Med* 1996;**89**:723-6.
66. Miller ME, Sals J, Kaye R, Levitsky LC. A familial plasma associated defect of phagocytosis: a new cause of recurrent bacterial infection. *The Lancet* 1968;**2**:60-3.
67. Pollard A, Galassini R, Rouppe van der Voort E, Hibberd ML, Booy R, Langford P *et al*. Cellular Immune Responses to *Neisseria meningitidis* in Children. *Infection and immunity* 1999;**67**:2452-63.
68. Hibberd ML, Sumiya M, Summerfield JA, Booy R, Levin M. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. *The Lancet* 1999;**353**:1049-53.
69. Aittoniemi J, Rintala E, Miettinen A, Soppi E. Serum mannan-binding lectin (MBL) in patients with infection: clinical and laboratory correlates. *APMIS* 1998;**105**:617-22.
70. Garred P, Madsen HO, Svejgaard A, Michaelsen TE. Mannose binding lectin and meningococcal disease. *The Lancet* 1999;**354**:336.
71. Hughes WT. The Athlete: An Immunocompromised Host. *Adv Pediatr Infect Dis* 1997;**13**:79-99.
72. Fitzgerald L. Overtraining Increases the Susceptibility to Infection. *Int.J.Sports Med.* 1991;**12**:S5-S8.
73. Brines R, Hoffman-Goetz L, Pederson BK. Can you exercise to make your immune system fitter? *Trends Immunology Today* 1996;**17**:252-4.

74. Yusuf HR, Rochat RW, Baughman WS, Gargiullo PM, Perkins BA, Brantley MD *et al.* Maternal cigarette smoking and invasive meningococcal disease: A cohort study among young children in metropolitan Atlanta, 1989 - 1996. *American Journal of Public Health* 1999;**89**:712-7.
75. Fischer M, Hedberg K, Cardoso P, Plikaytis B, Hoesly FC, Steingart KR *et al.* Tobacco smoke as a risk factor for meningococcal disease. *Pediatric Infectious Disease* 1997;**16**:979-83.
76. Stuart JM, Cartwright K, Robinson PM, Noah ND. Effect of smoking on meningococcal carriage. *The Lancet* 1989;723-5.
77. Blackwell CC, Tzanakaki G, Kremastinou J, Weir DM, Vakalis N, Elton RA *et al.* Factors affecting carriage of *Neisseria meningitidis* among Greek military recruits. *Epidem. Inf.* 1992;**108**:441-8.
78. Thomas JC, Bendana NS, Waterman SH, Rathbun M, Arakere G, Frasch CE *et al.* Risk Factors for Carriage of Meningococcus in the Los Angeles County Men's Jail System. *American Journal of Epidemiology* 1991;**133**:286-95.
79. Block C, Gdalevich M, Buber R, Ashkenazi I, Keller N. Factors associated with pharyngeal carriage of *Neisseria meningitidis* among Israel Defence Force personnel at the end of their compulsory service. *Epidem. Inf.* 1999;**122**:51-7.
80. Riordan T, Cartwright K, Andrews NJ, Stuart JM, Burris A, Fox AJ *et al.* Acquisition and carriage of meningococci in marine commando recruits. *Epidem. Inf.* 1998;**121**:495-505.
81. Blackwell CC, Weir DM, James VS, Todd WTA, Banatvala N, Chaudhuri AKR *et al.* Secretor status, smoking and carriage of *Neisseria meningitidis*. *Epidem. Inf.* 1990;**104**:203-9.
82. Fitzpatrick PE, Salmon RL, Hunter PR, Roberts RJ, Palmer S. Risk Factors for Carriage of *Neisseria meningitidis* during an outbreak in Wales. *Emerging Infectious Diseases* 2000;**6**:65-9.
83. Davies AL, O'Flanagan D, Salmon RL, Coleman TJ. Risk factors for *Neisseria meningitidis* carriage in a school during a community outbreak of meningococcal infection. *Epidem. Inf.* 1996;**117**:259-66.
84. McCall BJ, Neill AS, Young MM. Risk factors for invasive meningococcal disease in southern Queensland, 2000-2001. *Internal Medicine Journal* 2004;**34**:464-8.

85. Kriz P, Bobak M, Kriz B. Parental smoking, socioeconomic factors, and risk of invasive meningococcal disease in children: a population based case-control study. *Arch.Dis.Child* 2000;**83**:117-21.
86. Dye JA, Adler KB. Effects of cigarette smoke on epithelial cells of the respiratory tract. *Thorax* 1994;**49**:825-34.
87. Green GM, Carolin D. The depressant effect of cigarette smoke on the *in vitro* antibacterial activity of alveolar macrophages. *New England Journal of Medicine* 1967;**276**:421-7.
88. Raman AS, Swinburne AJ, Fedullo AJ. Pneumococcal adherence to the buccal epithelial cells of cigarette smokers. *Chest* 1983;**83**:23-7.
89. Cosio FG, Hoidal JR, Douglas SD, Michael AF. Binding of soluble immune complexes by human monocytes and pulmonary macrophages: effects of cigarette smoking. *J Lab Clin Med* 1982;**100**:469-76.
90. Pedreira FA, Guandolo VL, Feroli EJ, Mella GW, Weiss IP. Involuntary smoking and incidence of respiratory illness during the first year of life. *Pediatrics* 1985;**75**:594-7.
91. Murray M, Swan AV, Bewley BR, Johnson MRD. The development of smoking during adolescence - The MRC/Derbyshire smoking study. *Int J Epidemiol* 1983;**12**:185-92.
92. Turner-Warwick, M, Clarke, S. W, Woodcock, A. A, Bewley, B. R, Catford, J. C, Channer, K. S, Charlton, A, Couriel, J. M, Dunmore, J, Gillies, P. A, McNeill, A. D, Rees, P. J, Reid, D. J, Simpson, D, Stewart-Brown, S. L, Warner, J. O, Watkeys, J. E. M, and Harvey, F. A. H. Smoking and the young. 352-354. 1992. Journal of the Royal College of Physicians of London. College reports.
93. Galt M, Gillies P, Scott A. Smoking prevalence among 15-16 year olds in Doncaster, England. *J Pub Health Med* 1994;**16**:172-8.
94. Stanton WR, McClelland M, Elwood C, Ferry D, Silva PA. Prevalence, reliability and bias of adolescents' reports of smoking and quitting. *Addiction* 1996;**91**:1705-14.
95. Rawbone RG, Guz A. Cigarette smoking among secondary schoolchildren. *Arch.Dis.Child* 1982;**57**:352-8.
96. Williams R, Gillies P. Do we need objective measures to validate self-reported smoking? *Publ Hlth* 1984;**98**:294-8.

97. Rush B. An enquiry into the effects of ardent spirits upon the human body and mind with an account of the means of preventing and of the remedies for curing them. *G J Stud alcohol* 1943;**4**:321-41.
98. Imrey PB, Jackson LA, Ludwinski PH, England AC1, Fella GA, Fox BC *et al.* Meningococcal Carriage, Alcohol Consumption and Campus Bar Patronage in a Serogroup C Meningococcal Disease Outbreak. *J Clin Microbiol* 1995;**33**:3133-7.
99. Cookson ST, Corrales JL, Lotero JO, Regueira M, Binsztein N, Reeves MW *et al.* Disco fever: Epidemic meningococcal disease in Northeastern Argentina associated with disco patronage. *J.Inf.Diseases* 1998;**178**:266-9.
100. Jelfs J, Jalaludin B, Munro R, Patel M, Kerr M, Daley D *et al.* A cluster of meningococcal disease in Western Sydney, Australia initially associated with a nightclub. *Epidem.Inf.* 1998;**120**:263-70.
101. MacGregor RR. Alcohol and immune defense. *Journal American Medical Association* 1986;**256**:1474-81.
102. Smith FE, Palmer DL. Alcoholism, infection and altered host defenses: A review of clinical and experimental observations. *J Chron Dis* 1978;**29**:35-49.
103. Ben-Eliyahu S, Page GG, Yirmiya R, Taylor AN. Acute alcohol intoxication suppresses natural killer cell activity and promotes tumor metastasis. *Nat Med* 1996;**2**:457-60.
104. Bounds W, Betzing KW, Stewart RM, Holcombe RS. Social Drinking and the Immune Response: Impairment of Lymphokine-Activated Killer Activity. *The American Journal of the Medical Sciences* 1994;**307**:391-5.
105. May C. A burning issue? adolescent alcohol use in Britain 1970-1991. *Alcohol Alcohol* 1992;**27**:109-15.
106. Miller PM, Plant M. Drinking, smoking, and illicit drug use among 15 and 16 year olds in the United Kingdom. *BMJ* 1996;**313**:994-7.
107. Klein TW, Newton C, Friedman H. Cannabinoid receptors and immunity. *Immunology Today* 1998;**19**:373-81.
108. Glover, J. A. Observations of the meningococcus carrier rate and their application to the prevention of cerebrospinal fever. 50, 133-165. 1920. London, Medical Research Council.
109. Lowry S. Noise, space and light. *BMJ* 1989;**299**:1439-42.

110. Greenwood BM, Greenwood AM, Bradley AK, Williams K, Hassan-King M, Shenton FC *et al.* Factors influencing susceptibility to meningococcal disease during an epidemic in The Gambia, West Africa. *J.Infect.* 1987;**14**:167-84.
111. Zangwill KM, Schuchat A, Riedo FX, Pinner RW, Koo DT, Reeves MW *et al.* School-based Clusters of Meningococcal Disease in the United States. *Journal American Medical Association* 1997;**277**:389-95.
112. Kaiser AB, Hennekens CH, Saslaw MS, Hayes PS, Bennett JV. Seroepidemiology and chemoprophylaxis of disease due to sulphonamide-resistant *Neisseria meningitidis* in a civilian population. *Journal of Infectious Diseases* 1974;**130**:217-24.
113. Jones IR, Urwin G, Feldman RA, Banatvala N. Social deprivation and bacterial meningitis in North East Thames region: three year study using small area statistics. *BMJ* 1997;**314**:794-5.
114. Hudson PJ, Vogt RL, Heun EM, Brondum J, Coffin RR, Plikaytis BD *et al.* Evidence for school transmission of *Neisseria meningitidis* during a Vermont outbreak. *Pediatric Infectious Disease* 1986;**5**:213-7.
115. Feigin RD, Baker CJ, Herwaldt LA, Lampe RM, Mason EO, Whitney SE. Epidemic meningococcal disease in an elementary school classroom. *N.Engl.J.Med.* 1982;**307**:1255-7.
116. Morrow HW, Slaten DD, Reingold AL, Werner SB, Fenstersheib MD. Risk factors associated with a school-related outbreak of serogroup C meningococcal disease. *Pediatric Infectious Disease* 1990;**9**:394-8.
117. Neal K, Nguyen-Van-Tam JS, Monk P, O'Brien S, Stuart JM, Ramsay M. Invasive meningococcal disease among university undergraduates: association with universities providing relatively large amounts of catered hall accommodation. *Epidem.Inf.* 1999;**122**:351-7.
118. Harrison LH, Dwyer DM, Maples CT, Billmann L. Risk of Meningococcal Infection in College Students. *Journal American Medical Association* 1999;**281**:1906-10.
119. Robinson P, Taylor K, Nolan T. Risk factors for meningococcal disease in Victoria, Australia in 1997. *Epidem.Inf.* 2001;**127**:261-8.
120. Paffenbarger RS, Wing AL, Hyde RT. Physical activity as an index of heart attack risk in college alumni. *American Journal of Epidemiology* 1978;**108**:161-75.
121. Morse LJ, Bryan JA, Murle JP. The Holy Cross College football team hepatitis outbreak. *Journal American Medical Association* 1972;**219**:706-8.

122. Weinstein L. Poliomyelitis - a persistent problem. *New England Journal of Medicine* 1973;**288**:370-2.
123. Alexander JP, Chapman LE, Pallansch MA, Stephenson WT, Torok TJ, Anderson LJ. Coxsackievirus B2 Infection and Aseptic Meningitis: A Focal Outbreak among Members of a High School Football Team. *J.Inf.Diseases* 1993;**167**:1201-5.
124. Nieman DC, Henson DA, Gusewitch G. Physical activity and immune function in elderly women. *Medical Scientist Sports Medicine Physical Fitness* 1993;**29**:823-31.
125. Nieman DC, Johanssen LM, Lee JW. Infectious episodes in runners before and after the Los Angeles marathon. *Journal Sports Medicine Physical Fitness* 1990;**30**:316-38.
126. Nigg CR, Courneya KS. Transtheoretical Model: Examining Adolescent Exercise Behaviour. *Journal of Adolescent Health* 1998;**22**:214-24.
127. Goodman RA, Thacker SB, Solomon SL. Infectious diseases in competitive sports. *JAMA* 1994;**271**:862-7.
128. Heyderman R, Ben-Shlomo Y, Brennan CA, Somerset M. The incidence and mortality for meningococcal disease associated with area deprivation: an ecological study of hospital episode statistics. *Arch.Dis.Child* 2004;**89**:1064-8.
129. Williams CJ, Willocks LJ, Lake IR, Hunter PR. Geographic correlation between deprivation and risk of meningococcal disease: an ecological study. *BMC Public Health* 2004;**4**:30.
130. Stuart JM, Middleton N, Gunnell DJ. Socioeconomic inequality and meningococcal disease. *Communicable Disease Public Health* 2003;**6**:68.
131. Fone DL, Harries JM, Lester N, Nehaul L. Meningococcal disease and social deprivation: a small area geographical study in Gwent, UK. *Epidem.Inf.* 2003;**130**:53-8.
132. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *The Lancet* 1993;**341**:938-40.
133. Barker DJ. Fetal origins of coronary heart disease. *BMJ* 1995;**311**:171-4.
134. Barker DJ. Fetal origins of cardiovascular disease. *Annals of Medicine* 1999;**31**:3-6.

135. Read JS, Clemens JD, Klebanoff MA. Moderate Low Birth Weight and Infectious Disease Mortality during Infancy and Childhood. *American Journal of Epidemiology* 1994;**140**:721-33.
136. Yuan W, Basso O, Sorensen HT, Olsen J. Indicators of fetal growth and infectious disease in childhood - a birth cohort with hospitalization as outcome. *European Journal of Epidemiology* 2001;**17**:829-34.
137. McDade TW, Beck MA, Kuzawa C, Adair LS. Prenatal undernutrition, postnatal environments and antibody response to vaccination in adolescence. *American Journal of Clinical Nutrition* 2001;**74**:543-8.
138. Sorensen HT, Labouriau R, Jensen ES, Mortensen PB, Schonheyder HC. Fetal growth, maternal prenatal smoking, and risk of invasive meningococcal disease: a nationwide case-control study. *Int J Epidemiol* 2004;**33**:816-20.
139. McCullough ME, Hoyt WE, Larson DB, Koenig HG, Thoresen C. Religious involvement and mortality: a meta-analytic review. *Health Psychology* 2000;**19**:211-22.
140. Koenig HG, Cohen HJ, George LK, Hays JC, Larson DB, Blazer DG. Attendance at religious services, interleukin-6, and other biological parameters of immune function in older adults. *International Journal of Psychiatry Medicine* 1997;**27**:233-50.
141. Krucoff MW, Crater SW, Gallup D, Blankenship JC, Cuffe M, Guarneri M *et al.* Music, imagery, touch and prayer as adjuncts to interventional cardiac care: the Monitoring and Actualisation of Noetic Trainings (MANTRA) 11 randomised study. *The Lancet* 2005;**366**:211-7.
142. Sternberg EM. Emotions and disease: From balance of humors to balance of molecules. *Nat Med* 1997;**3**:264-7.
143. Biondi M, Zannino LG. Psychological Stress, Neuroimmunomodulation, and Susceptibility to Infectious Diseases in Animals and Man: A Review. *Psychotherapy and Psychosomatics* 1997;**66**:3-26.
144. Holmes TH, Rahe RH. The social readjustment rating scale. *Journal of Psychosomatic Research* 1967;**11**:213-8.
145. Rahe RH, Lind E. Psychosocial factors and sudden cardiac death: A pilot study. *Journal of Psychosomatic Research* 1971;**15**:19-24.
146. Theorell T, Rahe RH. Psychosocial factors and myocardial infarction 1: An inpatient study in Sweden. *Journal of Psychosomatic Research* 1971;**15**:25-31.

147. Cohen S, Williamson GM. Stress and Infectious Disease in Humans. *Psychological Bulletin* 1991;**109**:5-24.
148. Jemmott JB1, Locke SE. Psychosocial factors, Immunological mediation, and human susceptibility to infectious disease: How much do we know? *Psychological Bulletin* 1984;**95**:78-108.
149. Selye H. A syndrome produced by diverse nocuous agents. *Nature* 1936;**138**:132.
150. Dorn LD, Chrousos GP. The endocrinology of stress and stress system disorder in adolescence. *Endocrinology and Metabolism Clinics of North America* 1993;**22**:685-700.
151. Barglow P, Hatcher R, Edidin DV, Sloan Rossiter D. Stress and metabolic control in diabetes; psychosomatic evidence and evaluation of methods. *Psychosomatic Medicine* 1984;**46**:127-44.
152. Griffith LS, Field BJ, Lustman PJ. Life stress and social support in diabetes: association with glycemic control. *International Journal of Psychiatry Medicine* 1990;**20**:365-72.
153. Baldwin DR, Harris SM, Chambliss LN. Stress and illness in adolescence: Issues of race and gender. *Adolescence* 1997;**32**:839-53.
154. Susman EJ, Nottelman ED, Dorn LD, Inoff-Germain G, Chrousos GP. Physiological and behavioural aspects of stress in adolescence. *Advances Experimental Medical Biology* 1988;**245**:341-53.
155. Agnew R. Foundation for a general strain theory of crime and delinquency. *Criminology* 1992;**30**:47-87.
156. Agnew R. Stability and change over the life course: A strain theory explanation. New Brunswick: Transaction Publishers, 1997.
157. Compas BE, Davis GE, Forsythe CJ. Characteristics of life events during adolescence. *Am J Comm Psychol* 1985;**13**:677-91.
158. Agnew R, White HR. An empirical test of general strain theory. *Criminology* 1992;**30**:475-99.
159. Byrne DG, Byrne AE, Reinhart MI. Personality, stress and the decision to commence cigarette smoking in adolescence. *Journal of Psychosomatic Research* 1995;**39**:53-62.

160. Hawkins NG, Davies R, Holmes TH. Evidence for psychosocial specificity in pulmonary tuberculosis. *Trends Nature Tuberculosis Annals* 1955;**75**:780.
161. Holmes TH, Hawkins NG, Bowerman CE, Clarke ER, Joffe JR. Psychosocial and physiological studies of tuberculosis. *Psychosomatic Medicine* 1957;**19**:134-43.
162. Somers AR. Marital status, health, and use of health services: An old relationship revisited. *Journal American Medical Association* 1979;**241**:1818-22.
163. Lynch J. *The Broken Heart*. New York: Basic Books, 1977.
164. Craig, Lin. Mortality among elderly psychiatric patients: Basis for preventive intervention. *Journal of American Geriatric Society* 1981;**29**:181-5.
165. Boyce WT, Jensen EW, Cassel JC, Collier AM, Smith AH, Ramey CT. Influence of Life Events and Family Routines on Childhood Respiratory Tract Illness. *Pediatrics* 1977;**60**:609-15.
166. Cobb JMT, Steptoe A. Psychosocial stress and susceptibility to upper respiratory tract illness in an adult population sample. *Psychosomatic Medicine* 1996;**58**:404-12.
167. Cohen S, Tyrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. *New England Journal of Medicine* 1991;**325**:606-12.
168. Chester AC. Psychological stress and the common cold. *New England Journal of Medicine* 1992;**326**:644-6.
169. Wolf S. Reactions in the nasal mucosae: Relation of life stress to chronic rhinitis and sinus headache. *Archives of Otolaryngology* 1954;**59**:461-75.
170. Broadbent DE, Broadbent MHP, Phillpotts RJ, Wallace J. Some further studies on the prediction of experimental colds in volunteers by psychological factors. *Journal of Psychosomatic Research* 1984;**28**:511-23.
171. Cluff LE, Canter A, Imboden JB. Asian influenza: Infectious disease and psychological factors. *Arch Intern Med* 1966;**117**:159-63.
172. Belfer ML, Shader RI, Mascio AD, Harmatz JS, Nahum JP. Stress and bronchitis. *BMJ* 1968;**3**:805-6.
173. Locke SE, Heisel JS. The influence of stress and emotions on the human immune response. *Biofeedback Self-Regulation* 1977;**2**:320.

174. Greene WA, Betts RF, Ochitill HN, Iker HP, Douglas RG. Psychosocial factors and immunity: Preliminary report. *Psychosomatic Medicine* 1978;**40**:87.
175. Clover RD, Abell T, Becker LA, Crawford S, Ramsey JCN. Family functioning and stress as predictors of influenza B infection. *Journal of Family Practitioners* 1989;**28**:535-9.
176. Glaser R, Kiecolt-Glaser JK, Spiecher CE, Holliday JE. Stress, loneliness and changes in herpes virus latency. *Journal of Behavioural Medicine* 1985;**8**:249-60.
177. Kiecolt-Glaser JK, Dura JR, Spiecher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. *Psychosomatic Medicine* 1991;**53**:345-62.
178. Kiecolt-Glaser JK, Fisher LD, Ogrocki P, Stout JC, Spiecher CE. Marital quality, marital disruption and immune function. *Psychosomatic Medicine* 1987;**49**:13-34.
179. Kiecolt-Glaser JK, Glaser R, Shuttleworth EC, Dyer CS, Ogrocki P, Spiecher CE. Chronic stress and immunity in family caregivers of Alzheimer's disease victims. *Psychosomatic Medicine* 1987;**49**:523-35.
180. Kiecolt-Glaser JK, Kennedy S, Malkoff S, Fisher L, Spiecher CE. Marital discord and immunity in males. *Psychosomatic Medicine* 1988;**50**:213-29.
181. McKinnon W, Weisse C, Reynolds CP, Bowles CA, Baum A. Chronic stress, leukocyte subpopulations, and humoral response to latent viruses. *Health Psychology* 1989;**8**:389-402.
182. Kemeny ME, Cohen F, Zegans LA, Conant MA. Psychological and immunological predictors of genital herpes recurrence. *Psychosomatic Medicine* 1989;**51**:195-208.
183. Roark GE. Psychosomatic factors in the epidemiology of infectious mononucleosis. *Psychosomatics* 1971;**12**:402-11.
184. Glaser R, Pearson GR, Jones JF, Hillhouse J, Kennedy S, Mao H *et al.* Stress-related activation of Epstein-Barr virus. *Brain Behaviour Immunology* 1991;**5**:219-32.
185. Kiecolt-Glaser JK, Glaser R. Psychological influences on immunity: Making sense of the relationship between stressful life events and health. *Advances Experimental Medical Biology* 1988;**245**:237-47.

186. Kasl V, Evans AS, Niederman JC. Psychosocial risk factors in the development of infectious mononucleosis. *Psychosomatic Medicine* 1979;**41**:445-66.
187. Parkes CM, Brown RJ. Health after bereavement: A controlled study of young Boston widows and widowers. *Psychosomatic Medicine* 1972;**34**:449-61.
188. Meyer RJ, Haggerty RJ. Streptococcal infection in families: Factors altering susceptibility. *Pediatrics* 1962;**29**:539-49.
189. Cohen-Cole S, Cogen R, Stevens A, Kirk K, Gaitan E, Hain J *et al*. Psychosocial, endocrine, and immune factors in acute necrotizing ulcerative gingivitis ("trenchmouth"). *Psychosomatic Medicine* 1981;**43**:91.
190. Canter A. Changes in mood during incubation of acute febrile disease and the effects of pre-exposure psychological status. *Psychosomatic Medicine* 1972;**34**:424-30.
191. Rose RM. Endocrine responses to stressful psychological events. *The Psychiatric Clinics of North America* 1980;**3**:251-76.
192. Antonovsky A. Health, stress and coping. Washington: Jossey-Bass, 1979.
193. Cobb S. Social Support as a Moderator of Life Stress. *Psychosomatic Medicine* 1976;**38**:300-13.
194. Fiore J, Becker J, Coppel DB. Social Network Interactions: A Buffer or a Stress. *Am J Comm Psychol* 1983;**11**:423-39.
195. Ouellette Kobasa SC, Maddi SR, Puccetti MC, Zola MA. Effectiveness of hardiness, exercise and social support as resources against illness. *Journal of Psychosomatic Research* 1985;**29**:525-33.
196. Nuckolls KB, Cassel JC, Kaplan BH. Psychosocial assets, life crisis and the prognosis of pregnancy. *American Journal of Epidemiology* 1972;**95**:431-41.
197. Morris NM, Udry JR, Chase CL. Reduction of low birth weight rates by prevention of unwanted pregnancies. *American Journal of Public Health* 1973;**63**:935-8.
198. Holmes TH, Joffe JR, Ketcham JW. Experimental study of prognosis. *Journal of Psychosomatic Research* 1961;**5**:235-52.

199. de Araujo G, van Arsdell PP, Holmes TH, Dudley DL. Life change, coping ability and chronic intrinsic asthma. *Journal of Psychosomatic Research* 1973;**17**:359-63.
200. Sarason IG, Levine HM, Basham RB, Sarason BR. Assessing social support: The social support questionnaire. *Journal of Personality and Social Psychology* 1988;**44**:127-39.
201. Gore S. The effect of social support in moderating the health consequences of unemployment. *Journal of Health and Social Behaviour* 1978;**19**:157-65.
202. Hoffman JP. Investigating the age effects of family structure on adolescent marijuana use. *Journal of Youth and Adolescence* 1994;**23**:215-35.
203. Hoffman JP, Su SS. Stressful life events and adolescent substance use and depression: Conditional and gender differentiated effects. *Substance Use & Misuse* 1998;**33**:2219-62.
204. Shulman S. Close relationships and coping behaviour in adolescence. *Journal of Adolescence* 1993;**16**:267-83.
205. Rowlison RT, Felner RD. Major life events, Hassles, and Adaptation in Adolescence: Confounding in the Conceptualization and Measurement of Life Stress and Adjustment Revisited. *Journal of Personality and Social Psychology* 1988;**55**:432-44.
206. Brown JD, Siegel JM. Exercise as a buffer of life stress: A prospective study of adolescent health. *Health Psychology* 1988;**7**:341-53.
207. Brown JD, Lawton M. Stress and well-being in adolescence: The moderating role of physical exercise. *Journal of Human Stress* 1986;**12**:125-31.
208. Lamb KL, Brodie DA. The Assessment of Physical Activity by Leisure-Time Physical Activity Questionnaires. *Sports Medicine* 1990;**10**:159-80.
209. Baecke JAH, Burema J, Frijters JER. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 1982;**36**:936-42.
210. LaPorte RE, Montoye HJ, Caspersen CJ. Assessment of Physical Activity in Epidemiological Research: Problems and Prospects. *Public Health Reports* 1985;**100**:131-46.
211. Pate RR. Physical Activity Assessment in Children and Adolescents. *Critical Reviews in Food Science and Nutrition* 1993;**33**:321-6.

212. Weston AT, Petosa R, Pate RR. Validation of an instrument for measurement of physical activity in youth. *Med Sci Sports Exerc* 1997;**29**:138-43.
213. Melanson EL, Freedson PS. Physical Activity Assessment: A Review of Methods. *Critical Reviews in Food Science and Nutrition* 1996;**36**:385-96.
214. Taylor HL, Jacobs DRJ, Schucker B, Knudson J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. *J Chron Dis* 1978;**31**:741-55.
215. Mitchell JH, Haskell WL, Raven PB. Classification of Sports. *JACC* 1994;**24**:845-66.
216. Bernstein M, Sloutskis D, Kumanyika S, Sparti A, Schutz Y, Morabia A. Data-based Approach for Developing a Physical Activity Frequency Questionnaire. *American Journal of Epidemiology* 1998;**147**:147-54.
217. Ainsworth BE, Haskell WL, Leon AS, Jacobs DRJ, Montoye HJ, Sallis JF *et al.* Compendium of Physical Activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993;**25**:71-80.
218. Paykel ES, Prusoff BA, Uhlenhuth EH. Scaling of life events. *Archives of General Psychiatry* 1971;**25**:340-7.
219. Kashani JH, Vaidya AF, Soltys SM, Dandoy AC, Reid JC. Life events and major depression in a sample of inpatient children. *Compr Psych* 1990;**31**:266-74.
220. Sarason IG, Johnson JH, Siegel JM. Assessing the Impact of Life Changes: Development of the Life Experiences Survey. *Journal of Consulting and Clinical Psychology* 1978;**46**:932-46.
221. Jacobson AM, Rand LI, Hauser ST. Psychological stress and glycemic control: A comparison of patients with and without proliferative diabetic retinopathy. *Psychosomatic Medicine* 1985;**47**:372-81.
222. Cohen S, Wills TA. Stress, social support and the buffering hypothesis. *Psychological Bulletin* 1985;**98**:310-57.
223. Paykel ES. Methodological aspects of life events research. *Journal of Psychosomatic Research* 1983;**27**:341-52.
224. Raphael KG, Cloitre M, Dohrenwend BP. Problems of recall and misclassification with checklist methods of measuring stressful life events. *Health Psychology* 1991;**10**:62-74.

225. Bailey D, Garralda ME. The use of the social stress and support interview in families with deviant children: methodological issues. *Social Psychiatry* 1987;**22**:209-15.
226. Monck E, Dobbs R. Measuring life events in an adolescent population: methodological issues and related findings. *Psychological Medicine* 1985;**15**:841-50.
227. Coddington RD. The significance of life events as etiologic factors in the diseases of children - 2 A study of a normal population. *Journal of Psychosomatic Research* 1972;**16**:205-13.
228. Tennant C, Bebbington P, Hurry J. The role of life events in depressive illness: Is there a substantial causal relation? *Psychological Medicine* 1981;**11**:379-89.
229. Zimmerman M, O'Hara MW, Corenthal CP. Symptom contamination of life event scales. *Health Psychology* 1984;**3**:77-81.
230. McCubbin HI, Patterson JM, Bauman E, Harris LH. Adolescent-Family Inventory of Life Events and Changes. In McCubbin HI, Thompson A, eds. *Family assessment inventories for research and practice*, pp 89-100. Family Social Science, University of Minnesota: 1991.
231. Smith J, Harding S. Mortality of women and men using alternative social class classifications. *Health Inequalities*, pp 168-83. 1998.
232. Lewis G, Bebbington P, Brugha T, Farrell M, Gill B, Jenkins R *et al*. Socioeconomic status, standard of living and neurotic disorder. *The Lancet* 1998;**352**:605-8.
233. Pugh H, Power C, Goldblatt P, Arber S. Women's lung cancer mortality, socio-economic status and changing smoking patterns. *Social Science Medicine* 1991;**32**:1105-10.
234. Kopec JA, Esdaile JM. Bias in case- control studies. A review. *J Epidemiol Community Health* 1990;**44**:179-86.
235. Dyer C. The Gillick Judgement: Contraceptives and the under 16's: House of Lords ruling. *British Medical Journal (Clin Res Ed)* 1985;**291**:1208-9.
236. Abbott JD, Jones DM, Painter MJ, Young SEJ. The epidemiology of meningococcal infections in England and Wales 1912-83. *J Infect*. 1985;**11**:241-57.

237. Bell M. The Data Protection Act 1998. *Industrial Relations Law Bulletin* 1998;**605**:7-16.
238. McKennell. Bias in the Reported Incidence of Smoking by Children. *Int J Epidemiol* 1980;**9**:167-77.
239. Hebert JR, Clemow L, Pbert L, Ockene IS, Ockene JK. Social Desirability Bias in Dietary Self-Report May Compromise the Validity of Dietary Intake Measures. *Int J Epidemiol* 1995;**24**:389-98.
240. Fleming DM, Zambon M, Bartelds AI, de Jong JC. The duration and magnitude of influenza epidemics: a study of surveillance data from sentinel general practices in England, Wales and the Netherlands. *European Journal of Epidemiology* 1999;**15**:467-73.
241. Scanex. Formic 3 Automatic data capture system. 1999. United Kingdom.
242. Microsoft. Excel 4. 1997.
243. Stata 6.2 for windows. 2000. Stata Corp Ltd. College Station. Texas USA 77845.
244. Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley, 2000.
245. Stallard N, Tombs L. Including a time-of-year effect in the analysis of a matched case-control study. *Statistics in Medicine* 2004;**23**:3193-207.
246. CDR Weekly. *CDR Weekly* 2000;**10**.
247. Hennekens CH, Buring JE. Analysis of Epidemiological Studies: Evaluating the Role of Bias. In Mayrent SL, ed. *Epidemiology in Medicine*, pp 272-86. Philadelphia: Little,Brown, 1987.
248. Hennekens CH, Buring JE. Case-Control Studies. In Mayrent SL, ed. *Epidemiology in Medicine*, pp 132-52. Philadelphia: Little,Brown, 1987.
249. Nelson SJ, Charlett A, Orr HJ, Barker M, Neal K, Taylor C *et al*. Risk factors for meningococcal disease in university halls of residence. *Epidem.Inf.* 2001;**126**:211-7.
250. Neal K, Van-Tam J, Jeffrey N, Slack RCB, Madeley RJ, Ait-Tahar K *et al*. Changing carriage rate of *Neisseria meningitidis* among university students during the first week of term: cross sectional study. *BMJ* 2000;**320**:846-9.

251. Zaleski EH,.Schiaffino KM. Religiosity and sexual risk-taking behaviour during the transition to college. *Journal of Adolescence* 2000;**23**:223-7.
252. Bishop JP,.Stenger VJ. Retroactive prayer: lots of history, not much mystery, and no science. *BMJ* 2004;**329**:1444-6.
253. Schulz KF,.Grimes DA. Case-control studies: research in reverse. *The Lancet* 2002;**359**:431434-434.
254. Hennekens CH, Buring JE. Epidemiology in Medicine. Philadelphia: Little,Brown, 1987.
255. Bose A, Coen PG, Tully J, Viner R, Booy R. Effectiveness of meningococcal C conjugate vaccine in teenagers in England. *The Lancet* 2003;**361**:675-6.

Appendices

Appendix 1; Introductory letter to CCDC's

Dear Dr,

Re. Risk factors for meningococcal disease in adolescents

I am writing by way of introduction and to ask for your assistance.

I am a specialist paediatric registrar currently employed by the Institute of Child Health as clinical research fellow to oversee a multi-centre study to investigate risk factors for the development of meningococcal disease in adolescence. This study is to run for eighteen months from January 1999.

I write to request your help in identifying cases to be included in the study and have enclosed a summary of the study protocol. I would be more than happy to provide you with the full protocol on request. The study has been funded by the Meningitis Research Foundation and addresses five main hypotheses surrounding the teenage peak in invasive meningococcal disease. It will also, we hope, be able to provide us with information about other behavioural and social factors of the adolescent that might be relevant to this disease.

For this study I need a system for early identification of young people between the ages of 15 and 19 years with suspected meningococcal disease (meningitis or septicaemia). These can then be confirmed or otherwise using laboratory criteria at a later date. The reason for the urgency is the need to collect an acute respiratory sample for viral studies. The association that we are attempting to demonstrate, between a range of respiratory viruses and meningococcal disease, relies on being able to get a sample as rapidly as possible. This means identifying possible cases within 48 hours of admission.

The study has the full support of the Executive of the Public Health Medicine Environmental Group and has been approved by the North Thames Multi-Centre

Research Ethics Committee. It was discussed at the Trent CCDC December meeting. Local ethical approval has been applied for.

Although I appreciate how busy you are, I am writing to ask whether you would be willing to assist in this study. All that I would need is notification by telephone of the name, date of birth, consultant in charge and admitting hospital of a case notified to you as suspected or confirmed meningococcal disease between the ages of 15 and 19 years inclusive, as soon as was possible. I will be carrying a mobile telephone so that I will be easily and constantly contactable between 8am and 11pm every day. This does include weekends and so on-call cover may need to be aware of the study. In addition I will have an answering service on the office telephone where a message can be left. There are no questionnaires for you to fill in or data for you to collect, I just need a brief phone call. Your help will be acknowledged in any publications.

As I will also be updated daily with information on all samples in the relevant age group received by the Manchester Reference Unit, I can offer to inform you of all the cases that you may not have heard about via clinicians.

In addition we will be collecting data on respiratory viruses in this population, and although these results will not be available for some months following admission of the case, I would be more than happy to advise you of the results of these investigations.

I would like to thank you in advance for being prepared to help with this study and if there are questions that you want answered I will be contacting you by telephone in the near future to discuss the study with you. However if you would like any information in the interim, please feel free to contact me.

An aide-memoire for the office wall is enclosed should you wish to use it.

I look forward to speaking to you soon,

Yours Sincerely,

Dr Jo Tully, Clinical Research Fellow

Appendix 2; Introductory letter to consultants

Dear Dr,

I am writing to inform you about a study, funded by the Meningitis Research Foundation that is underway in the UK and which may involve patients admitted under your care.

This multi-centre study is taking place across six regions of England and aims to investigate risk factors for the development of meningococcal disease in the adolescent population. Social, biological and psychological factors that are specific to this age group and that may predispose to the development of this serious disease will be explored. A summary of the study protocol is enclosed.

We will be recruiting 15 to 19 year olds who have recently developed meningococcal disease. Some of the local consultants in communicable diseases may notify the research centre of cases of suspected meningococcal disease in this age group that they are made aware of, but we are very keen to develop a second system for notification of cases. CCDC's are not always informed of cases in time.

I am writing to ask whether you would be willing to allow your staff to let us know if you have a case of meningococcal disease in this age group admitted under your care. All we require is a brief 'phone call as soon as possible after admission of the patient and messages can be left on either number 24 hours a day. I have enclosed some small posters for the ward staff which may serve to act as reminders.

The vast majority of patients admitted to hospital with this diagnosis will require serial blood test monitoring as part of routine clinical management. We need to test the subjects for evidence of recent viral infection and this will necessitate taking a single blood sample and a viral throat swab early in the course of admission. As our research centre is situated in London, it will be necessary to involve clinical staff in the collection of this sample. A questionnaire will be delivered to all subjects after discharge from hospital and controls will be selected from GP lists.

I will be liaising with consultant staff as soon as I am made aware of the admission of the case in order to gain permission to recruit the case and will make arrangements for transport of samples to our central laboratory. It will be possible for results to be made available to clinical staff after a period of time.

We have obtained ethical approval for this study from the Multi-centre Research Ethics Committee, and local ethical approval has been sought. A letter has been sent to the Medical Director of your trust. Data collection started on January 4th 1999.

I hope that you have no objections to this study taking place with the possible involvement of your patients and that you would be able to distribute this information amongst senior ward staff.

Thanking you in advance for your assistance. It is much appreciated.

If you have any questions about the study please do not hesitate to contact me.

Yours sincerely,

Dr Jo Tully

Clinical Research Fellow

Appendix 3; Introductory letter to medical directors

Dear

I am writing to inform you about a study, funded by the Meningitis Research Foundation that is taking place in the UK and which may involve patients from your Trust.

This multi-centre study is taking place across six regions of England and aims to investigate risk factors for the development of meningococcal disease in the adolescent population. Social, biological and psychological factors that are specific to this age group and that may predispose to the development of this serious disease will be explored.

We will be recruiting 15 to 19 year olds who have recently developed meningococcal disease. The consultants in communicable diseases will notify the research centre of all cases of suspected meningococcal disease in this age group. Once notified of a case, the consultant in charge will be approached for permission to involve their patient.

The vast majority of patients admitted to hospital with this diagnosis will require serial blood test monitoring as part of routine clinical management. We need to test the subjects for evidence of recent viral infection and this will necessitate taking a single blood sample and a viral throat swab early in the course of admission. As our research centre is situated in London, it will be necessary to involve clinical staff in the collection of this sample. A questionnaire will be delivered to all subjects after discharge from hospital and controls will be selected from GP lists.

I will be liaising with medical staff as soon as I am made aware of the admission of the case and will make arrangements for transport of samples to our central laboratory. It will be possible for results to be made available to clinical staff after a period of time.

We have obtained ethical approval for this study from the Multi-centre Research Ethics Committee, and local ethical approval has been sought.

We started data collection on January 4th 1999.

I hope that you have no objections to this study taking place with the possible involvement of your patients and that you can support the involvement of clinical staff in sample collection. I would be most grateful if you could bring this study to the attention of senior physicians in adult and paediatric services.

If you have any questions about the study please do not hesitate to contact me,

Yours sincerely,

Dr Jo Tully

Clinical Research Fellow

Appendix 4; Introductory letter to Intensive Care Units

Dear Dr

I am writing to inform you about a study, funded by the Meningitis Research Foundation that is underway in the UK and which may involve patients admitted to your unit.

This multi-centre study is taking place across six regions of England and aims to investigate risk factors for the development of meningococcal disease in the adolescent population. Social, biological and psychological factors that are specific to this age group and that may predispose to the development of this serious disease will be explored. A study protocol is enclosed.

We will be recruiting 15 to 19 year olds who have recently developed meningococcal disease. The local consultants in communicable diseases will notify the research centre of all cases of suspected meningococcal disease in this age group that they are made aware of, but we are very keen to develop a second system for notification of cases. CCDC's are not always informed of cases in time.

I am writing to ask whether you would be willing to allow your ITU staff to let us know if you have a case of meningococcal disease in this age group admitted to your unit. All we require is a brief 'phone call as soon as possible after admission and messages can be left on either number 24 hours a day. I have enclosed a small poster which serves to act as a reminder.

The vast majority of patients admitted to hospital with this diagnosis will require serial blood test monitoring as part of routine clinical management. We need to test the subjects for evidence of recent viral infection and this will necessitate taking a single blood sample and a viral throat swab early in the course of admission. As our research centre is situated in London, it will be necessary to involve clinical staff in the collection of this sample. A questionnaire will be delivered to all subjects after discharge from hospital and controls will be selected from GP lists.

I will be liaising with consultant staff as soon as I am made aware of the admission of the case in order to gain permission to recruit the case and will make arrangements for transport of samples to our central laboratory. It will be possible for results to be made available to clinical staff after a period of time.

We have obtained ethical approval for this study from the Multi-centre Research Ethics Committee, and local ethical approval is being sought. A letter has been sent to the Medical Director of your trust. Data collection started on January 4th 1999.

I hope that you have no objections to this study taking place with the possible involvement of your unit and that you can support the involvement of clinical staff in sample collection.

If you have any questions about the study please do not hesitate to contact me,

Yours sincerely,

Dr Jo Tully

Clinical Research Fellow

Appendix 5; Introductory letter to microbiologists

Dear Dr

I am a clinical research fellow at the Institute of Child Health in London. I have the responsibility for running a multi-centre case-control study to investigate the risk factors for the development of meningococcal disease amongst the adolescent population.

I am writing to inform you about the study. The Medical Directors of Trusts that may admit patients suitable for inclusion in the study have received a letter about the work and may already have passed this information over to you. However, it seems that many microbiology departments are still unaware that this study is happening. I would like to emphasise that we are not asking microbiologists for any direct assistance, but would like the department to be aware that patients might become involved.

A summary of the study protocol has been enclosed. We will be notified of possible cases as early in the course of admission as possible. Notification is via Consultants in Communicable Disease Control, individual wards or units or the Manchester Reference Laboratory.

A sample of blood and both a throat swab and pernasal swab will be collected acutely after gaining consent from the patient or the next-of-kin. This is sent to the Colindale Public Health Laboratory in North London for respiratory virus analysis. Further follow-up will be after discharge at the patient's home.

We have both North Thames Multi-centre Ethics Committee approval as well as local ethical approval.

I hope you find the protocol of interest. If you have any questions about the study, please do not hesitate to contact us on the numbers shown below.

Yours sincerely,

Dr Jo Tully, Clinical Research Fellow

Appendix 6; Introductory letter to GP

Dear Dr

I am a clinical research fellow at the Institute of Child Health. We are currently running a national study to examine the risk factors for development of meningococcal disease in adolescence and the efficacy of the new group C vaccine. Summarised study protocols are enclosed.

The study has been approved both by North Thames Multi-Centre Ethics Committee and by the local ethics committee.

The study centre has been notified of a case of meningococcal disease in the age group that we are interested in that is registered with you (shown below). In order to recruit a matched control to the study, we need to identify the 4 patients on your list *of the same sex* that have a date-of-birth closest to that of the unwell patient. This can be done using computerised records. Due to the nature of the study, we are attempting to recruit controls *within 2 weeks of admission of the case*.

Case name

Date of birth

Sex

I am writing to ask for your help. I have enclosed 4 sets of information sheets with a covering letter from yourself and stamped envelopes, and would be extremely grateful if you could forward these to the 4 controls that you have identified *as soon as possible*.

If it is necessary for us to contact these controls *once they have received the information from you*, and you are happy for us to do this, could the control identification form be completed and sent to us in the stamped addressed envelope provided.

If you have any queries about the study, please do not hesitate to contact me on the number shown below.

Yours sincerely,

Dr Jo Tully
Clinical Research Fellow

Appendix 7; Case notification form

Name

Date of Birth

Address & tel. no

Admitting hospital

Admitting Consultant

Notified By

Date & time notified

Tel of hospital

Fax no of department

Contact name/number of clinician

Date admitted

Ward admitted to

Name & tel no of GP

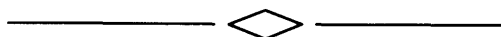
Appendix 8; Recruitment checklist

- Study number
- Received notification & filled in form with details ☐
- Faxed through documents to consultant in charge to gain permission ☐
- Contact with Registrar/consultant in charge of subject to gain cooperation ☐
- Obtained consent ☐
- Sent sample collection pack out to hospital with study number filled in ☐
- Organise return courier ☐
- E-mail Diana regarding case samples ☐
- Filled in 2 identifier notebooks ☐
- Posted documents to GP of case to get 4 controls with study no. filled in ☐
- Date posted.....
- Contacted GP to get 4 numbers if required ☐
- Post consultant & team thanks/ request for results ☐
- Date posted.....
- Contacted a control to get cooperation and arrange provisional date of interview ☐
- Posted standard CCDC thank you letter ☐
- Posted standard GP thank you letter ☐
- Contacted registrar to check samples taken and cases progress/survival ☐
- Contacted case at home to arrange interview date ☐

- Contacted control to arrange interview date ☐
- Reconfirmed dates and times with case and control ☐
- Interviewed cases and control and sent samples to lab ☐
- Sent case thanks ☐
- Sent control thanks ☐
- Entered data into database ☐

Appendix 9; General information sheet

TAKING PART IN RESEARCH



GENERAL INFORMATION FOR THOSE CONSIDERING TAKING PART IN RESEARCH

Study Title; Meningococcal disease in teenagers; Finding out why

You are being invited to take part in a research project. Here is some information to help you decide whether or not to take part. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything you do not understand or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

You may or may not receive any direct benefit from taking part in the study. However, information obtained during the course of the study may help us to understand better your condition or illness. It may also help us in selecting treatment for future patients.

It is up to you to decide whether to take part or not. If you do decide to take part you will be given an information sheet and consent form. Even if you do decide to take part, you are free to withdraw at any time and without giving a reason. This will not affect the standard of care you will receive. Your doctor will not be upset if you decide not to take part.

Depending upon the type of study, your GP will normally be informed that you are taking part. If this is a problem for you, you should discuss it with your study researcher.

Consumers for Ethics in Research (CERES) publish a leaflet entitled “Medical Research and You”. This leaflet gives more information about medical research and

looks at some questions you may want to ask. A copy may be obtained from CERES,
PO Box 1365, London N16 0BW.

If you have any questions, please contact the study doctor Dr Jo Tully on 0171 242
9789 Ext 2443 or 0171 905 2340

Appendix 10; Case information sheet

TAKING PART IN RESEARCH



INFORMATION FOR PATIENTS ABOUT THIS STUDY

Study Title; Meningococcal disease in teenagers: Finding out why

What is the purpose of the study?

Meningitis or septicaemia (blood poisoning) caused by the meningococcal germ (bacteria) is known as “meningococcal disease”. This is a life-threatening illness that particularly affects children and young people. Even if the illness is treated promptly, its effects can still be extremely serious.

Unfortunately meningococcal disease has become more common in this country especially among young people of your age.

In order to discover ways of preventing this serious disease, we first need to find out why it is that young people are at risk. In order to do this we need your help.

The study that we are running will take place across England over a two year period. We want to study young people like you who have had meningococcal disease, as well as people of the same age that haven't. We can then compare these two groups.

During the study we will be looking at things that we think might make a difference to whether or not you get meningococcal disease. The study will be covering topics such as your general health, the place where you live, the people that you live with, the kinds of things you like to do in your spare time and whether or not you suffer from stress. We will also look at the possibility of genetic factors being involved.

Why have I been chosen?

Because meningococcal disease is more common between the ages of 15 and 19 years, we are only studying people between these ages.

Because your doctor thinks that you might have meningococcal disease, a doctor in public health has been told about you. Either the public health doctor or the laboratory that looks at your test results informed the study centre that you are in hospital so that we might ask you to help us with our study.

We hope to ask about 200 other patients with meningococcal disease to help us with our study.

Who is organising the study?

The study has been organised by a group of doctors that work at the Institute of Child Health, London, St Mary's Hospital Paddington, Great Ormond Street Hospital, London and at the Central Public Health Laboratory in Colindale. The study is being funded by the Meningitis Research Foundation that provides money to help doctors and scientists find out about meningococcal disease.

What will happen to me if I take part?

If you decide to take part, we will ask you to sign a consent form. While you are in hospital you will be having blood tests, so we will ask the doctors looking after you if they can take a few teaspoonfuls of extra blood when they are doing other important tests. We will use this blood for our study. Some of you will have the blood tested to see if you were exposed to cigarette smoke just before you got ill. You will not have any extra needles while in hospital because of this study. We will also ask the nurses to take a swab from your throat and nose. This swab is to look for viruses that you may be carrying.

In about 2 weeks time we will contact you again and arrange to come to your home. At this visit you will be asked to have another blood test done and to fill in a confidential questionnaire. This will take about an hour.

The questionnaire includes questions about you and your lifestyle, some of which may be personal. You may not want to answer all the questions. If there are questions that you do not want to answer, then you do not have to do so.

After this is complete you will not have to be involved any further.

Are there any disadvantages in taking part in this study?

Taking part in this study will not affect the treatment you receive. There are no side-effects or serious risks involved in taking part. However you will have to have an extra blood test which can be uncomfortable. If you agree to take part you will be offered an anaesthetic cream for that blood test. This numbs the skin and stops it hurting.

We will also be asking you for an hour of your time to answer our questionnaire.

Remember, taking part is voluntary and you can change your mind at any stage.

What are the possible risks of taking part?

There are no risks from taking part in this study.

What are the possible benefits of taking part?

We are hoping that by finding out more about why young people are getting this illness, we may find ways of helping to prevent it.

Taking part in this study will not mean that you get better treatment than other people but it might help prevent people in the future from getting this disease.

We hope you may find it interesting and it will certainly help other people in your age group.

Is my doctor being paid for including me in the study?

No. Your doctor has agreed that you may take part, but he or she will not receive any payment for it.

Are there any restrictions on what I might eat or do?

No. You can eat anything and do whatever you would normally. This will not affect the results of your blood test.

Confidentiality – who will know I am taking part in the study?

You may feel worried about the information that you give us. Everything that you tell us will be treated as strictly confidential. This means that no-one else will be able to see this information, and that includes your parents and other relatives. Your name won't be on the information that we store on our computers, however we will keep a separate list of names so that if we want to talk to you in the future we will be able to contact you.

If you are in hospital at the moment, then we will inform your doctors that you are taking part. Your GP will know that you have agreed to participate but he or she will not know any of the results. All genetic tests will be done anonymously and results will not be available.

Occasionally it might be necessary to get some information about the treatment that you received during this illness from your medical records. All information collected in this way will also be kept strictly confidential.

MREC approval

This project has been approved by an independent research ethics committee who believe that it is of minimal risk to you. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

What will happen to the results of the study?

The results of this study will not be available for at least 2 years. It will not be possible for us to let you know the results of the study personally, but we hope that the information that we get will make a difference to many people.

We hope that the results of this study will be published in the medical literature.

Contact for further information

If you have any questions or worries at any time, then you can contact the study doctor, Dr Jo Tully on the number given below. If no-one is there to take your call you can leave a message and someone will call you back.

If you want any further advice on meningococcal disease or wish to talk to someone other than the study doctor, then you can call the Meningitis Research Foundation Helpline on 01454 413344 / 281 811 or speak to Linda Glennie on the same number.

Thank you very much for considering taking part in our research.

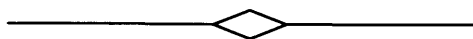
Dr Jo Tully, Clinical Research Fellow

Institute of Child Health and Great Ormond Street Hospital for Sick Children

Tel; 0171 242 9789 Ext 2443 or 0171 905 2340

Appendix 11; Control information sheet

TAKING PART IN RESEARCH



INFORMATION FOR PARTICIPANTS IN THIS STUDY

Study Title; Meningococcal disease in teenagers: Finding out why

What is the purpose of the study?

Meningitis or septicaemia (blood poisoning) caused by the meningococcal germ (bacteria) is known as “meningococcal disease”. You may have heard about this disease on the television or in the newspapers.

This is a life-threatening illness that particularly affects children and young people. Even if the illness is treated promptly, its effects can still be extremely serious. Unfortunately meningococcal disease has become more common in this country especially among young people of your age.

In order to discover ways of preventing this serious disease, we first need to find out why it is that young people are at risk. In order to do this we need your help.

The study that we are running will take place across England over a two year period. We are going to study a large group of people of your age who have had meningococcal disease recently. We need to compare this group of people with a group of people of the same age who haven’t had meningococcal disease. That’s where you come in.

During the study we will be looking at things that we think might make a difference to whether or not you get meningococcal disease. The study will be covering topics such as your general health, the place where you live, the people that you live with, the kinds of things you like to do in your spare time and whether or not you suffer from stress. We will also look at the possibility of genetic factors being involved.

Why have I been chosen?

Because meningococcal disease is more common between the ages of 15 and 19 years, we are only studying people between these ages.

We got your name from your GP who is collaborating with this study. Your date of birth shows that you are the right age to participate.

We hope to ask about 200 other people like you to take part in our study.

Who is organising the study?

The study has been organised by a group of doctors that work at the Institute of Child Health, London, St Mary's Hospital Paddington, Great Ormond Street Hospital, London and at the Central Public Health Laboratory in Colindale. The study is being funded by the Meningitis Research Foundation that provides money to help doctors and scientists find out about meningococcal disease.

What will happen to me if I take part?

If you decide to take part, the study doctor will arrange a convenient time at which she can visit your home. We will ask you to sign a consent form. At this visit you will be given a confidential questionnaire and have a blood test done. Some of you will have your blood tested to see whether you have been exposed to cigarette smoke. We will also take a swab from your nose and throat. This swab is to look for viruses that you might be carrying. This will all take about an hour.

The questionnaire includes questions about you and your lifestyle, some of which may be personal. You may not want to answer all the questions. If there are questions that you do not want to answer, then you do not have to do so.

After this is complete you will not have to be involved any further.

Are there any disadvantages in taking part in this study?

There are no side-effects or serious risks involved in taking part in this study. However you will have to have a blood test which can be uncomfortable and may

leave a bruise. If you agree to take part you will be offered an anaesthetic cream for that blood test. This numbs the skin and stops it hurting.

We will also be asking you for an hour of your time to answer our questionnaire.

Remember, taking part is voluntary and you can change your mind at any stage.

What are the possible risks of taking part?

There are no risks from taking part in this study.

What are the possible benefits of taking part?

We are hoping that by finding out more about why young people are getting this illness, we may find ways of helping to prevent it.

Taking part in this study might help prevent people in the future from getting this disease.

We hope you may find it interesting and will certainly help other people in your age group.

Is my doctor being paid for including me in the study?

No. Your doctor has agreed that you may take part, but he or she will not receive any payment for it.

Are there any restrictions on what I might eat or do?

No. You can eat anything and do whatever you would normally. This will not affect the results of your blood test.

Confidentiality – who will know I am taking part in the study?

You may feel worried about the information that you give us. Everything that you tell us will be treated as strictly confidential. This means that no-one else will be able to see this information, and that includes your parents and other relatives. Your name won't be on the information that we store on our computers, however we will keep a

separate list of names so that if we want to talk to you in the future we will be able to contact you.

Your GP will know that you have agreed to participate but he or she will not know any of the results. All genetic tests will be done anonymously and results will not be available.

MREC approval

This project has been approved by an independent research ethics committee who believe that it is of minimal risk to you. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or any manufacturer involved.

What will happen to the results of the study?

The results of this study will not be available for at least 2 years. It will not be possible for us to let you know the results of the study personally, but we hope that the information that we get will make a difference to many people.

We hope that the results of this study will be published in the medical literature.

Contact for further information

If you have any questions or worries at any time, then you can contact the study doctor, Dr Jo Tully on the number given below. If no-one is there to take your call you can leave a message and someone will call you back.

If you want any further advice on meningococcal disease or wish to talk to someone other than the study doctor, then you can call the Meningitis Research Foundation Helpline on 01454 413344 / 281 811 or speak to Linda Glennie on the same number.

Thank you very much for considering taking part in our research.

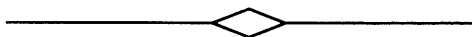
Dr Jo Tully, Clinical Research Fellow

Institute of Child Health and Great Ormond Street Hospital for Sick Children

Tel; 0171 242 9789 Ext 2443 or 0171 905 2340

Appendix 12; Relative information sheet

TAKING PART IN RESEARCH



INFORMATION FOR RELATIVES ABOUT OUR STUDY

Study Title; Meningococcal disease in teenagers: Finding out why

What is the purpose of this study?

Meningitis or septicaemia (blood poisoning) caused by the meningococcal germ (bacteria) is known as “meningococcal disease”. This is a life-threatening illness that particularly affects children and young people. Unfortunately meningococcal disease has become more common in this country especially among young people.

In order to discover ways of preventing this serious disease, we first need to find out why it is that young people are at risk.

We want to look at the kinds of things that might make young people more likely to get this disease. We are especially interested in finding out if viral infections like flu increase the risk.

What do you want me to do?

We understand that you have a relative who has meningococcal disease and that this is a very worrying time for you. In order for us to try and find out if your relative picked up a viral infection just before they got meningococcal disease we need a small sample of blood from them to use for our study. This will be taken at the same time as other blood tests, so no extra needles will be used. We also need a swab from their throat or nose to use for our study. This will be an extra sample from normal.

What will happen next?

If you agree to let us take these samples for our study, we will ask you to sign a consent form. After your relative gets better, our study doctor will contact them to ask if they would like to take part in the next stage of our study. That will involve answering some questions and having another small blood sample taken. Just because you have agreed to let us have the samples does not mean your relative has to agree to take part when they get better.

Further information

The doctors looking after your relative know about this study and support it. They can give you some information about the study. If you want more information, the study doctor will be very happy to answer your questions. You can contact her on the number given below. If there is no-one there to take your call, please leave a message and she will get back to you as soon as she can.

Dr Jo Tully, Clinical Research Fellow

Institute of Child Health and Great Ormond Street Hospital

Tel; 0171 242 9789 Ext 2443 or 0171 905 2340

Appendix 13; Case consent form

Subject number; □□□□

(This consent form is for the CASES)

CONSENT FORM

Title of Project: Meningococcal disease in teenagers; Finding out why

Name of Researcher: Dr Joanna Tully (Clinical Research Fellow)

Tel; 0171 2429789 Ext 2443

Please initial box

1. I confirm that I have read and understood the information sheet (version 1)

for the above study.

☐
2. I understand that my participation is voluntary and that I am free to withdraw

at any time without my medical care or legal rights being affected.

☐
3. I understand that I will be interviewed in confidence and that a blood test, throat

swab and nose swab may be taken.

☐
4. I am willing to allow access to my medical records if required but understand

that strict confidentiality will be maintained.

☐
5. I agree to take part in the above study.

☐

Name of subject

Date

Signature

Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____

Researcher	Date	Signature
_____	_____	_____

Please keep copy in medical notes

Appendix 14; Control consent form

Subject number: □□□□

(This consent form is for the CONTROL subjects)

CONSENT FORM

Title of Project: Meningococcal disease in teenagers; Finding out why

Name of Researcher: Dr Joanna Tully (Clinical Research Fellow)

Tel; 0171 2429789 Ext 2443

Please initial box

1. I confirm that I have read and understood the information sheet (version 2)

☐

 for the above study.
2. I understand that my participation is voluntary and that I am free to withdraw

☐

 at any time without my medical care or legal rights being affected.
3. I understand that I will be interviewed in confidence and that a blood test,

☐

 throat swab and nose swab will be taken.
4. I agree to take part in the above study.

☐

Name of subject	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____

1 copy to be retained by subject, 1 copy for researcher.

Appendix 15; Next-of-kin consent form

Subject Number

(This consent form is for the next of kin)

CONSENT FORM

Title of Project: Meningococcal disease in teenagers; Finding out why

Name of Researcher: Dr Joanna Tully (Clinical Research Fellow)

Tel; 0171 2429789 Ext 2443

Please initial box

I confirm that I have read and understood the attached information sheet ☐
for the above study.

I give my consent to a blood sample, throat swab and nose swab being taken ☐
and used for the purposes of this study.

I agree to my relative being contacted at a later stage to discuss further ☐
involvement in this study.

Name of next of kin	Date	Signature
---------------------	------	-----------

_____	_____	_____
-------	-------	-------

Name of person taking consent (if different from researcher)	Date	Signature
---	------	-----------

_____	_____	_____
-------	-------	-------

Researcher	Date	Signature
------------	------	-----------

1 copy to be retained by subject, 1 copy for medical notes.

Appendix 16; Instruction sheet

Taking Specimens For The Teenage Meningococcal Study

This study is looking at a possible association between respiratory viruses and the development of meningococcal disease. We need to collect both blood samples and a specimen for isolation of respiratory viruses (throat swab and pernasal swab).

Enclosed in this pack are the swabs and blood bottles that you will need.

We need a throat and pernasal swab placed in the special viral medium provided, as well as 8-10mls of blood in the plain bottle.

Taking a pernasal swab

Use the special soft wired sterile swab provided. This minimises trauma to the nasal tissue.

Holding the head upwards, pass the swab along the floor of the nasal cavity to the posterior wall of the nasopharynx.

Gently rotate and withdraw the swab.

Please cut the ends of the swabs off with normal scissors and place in the small bottle of specially-prepared medium provided for you in your pack. Please PUT BOTH SWAB ENDS IN THE SAME BOTTLE.

Please label them clearly with the patients details (name, date-of-birth and date of sample).

A form is enclosed. Please fill this in and don't forget to enclose it in the pack with the specimens.

Place the blood sample and swabs inside the special box provided (this is a legal requirement). The box can then be placed inside the pre-addressed grey courier bag.

We will liase with you to arrange a courier to pick the specimens up.

If there are any problems or you have any queries please phone Dr Jo Tully on 0171 905 2340 or 0171 242 9789 Ext 2443 or mobile number 07957 316172 (weekends or evenings).

Appendix 17; Checklist for GP pack

Teenage meningococcal study GP pack

Each pack sent to the GP should contain the following;

- Letter of introduction
- Summarised protocol
- 3 control information sheets
- 3 general information sheets
- 3 letters from GP to control with study numbers filled in
- 3 stamped un-addressed envelopes
- 3 stamped envelopes addressed to us
- Response form

Appendix 18; GP letter to control

Dear

We are looking for some young people to help out with a research study into teenagers and meningitis.

We are currently working with a research team from the Meningitis Research Foundation based at the Institute of Child Health in London. This research is looking at why young people get meningitis and how vaccines protect them. As your GP I have been asked to help the research team find a young person to help them with this research and your date of birth shows you are in the right age group to participate.

Information about this study is enclosed.

This is an important study that may help doctors to understand why young people of your age contract meningitis more commonly than other people. It is hoped that what we learn from this study will help us prevent young people getting this disease in the future.

The success of this study depends on the research doctor or nurse talking to someone like you as soon as possible. This will only take about an hour of your time and can be done in your own home.

If you are interested in taking part or want more information, would you please telephone either of the following numbers *as soon as possible, preferably today*, and speak to the study doctor or nurse. The numbers you can ring are 020-7242 9789 Extension 2443, or mobile number 07957 316172.

Alternatively would you please return the response sheet in the envelope provided. If you call, to help you to decide whether to take part the study doctor or nurse will be happy to explain anything that you haven't understood. If the study team don't hear from you in a few days time I have given them permission to contact you by telephone.

We hope that you will be interested in taking part in this study. The help of people like you is vital. If you do not want to take part, please let them know and they will not contact you again.

Yours sincerely,

Appendix 19; Control Identification Form

GP name.....

Study Number.....

Please ensure Name 1 receives pack 1 and so on...

Name 1:

Address:

Date of Birth:

Telephone Number:

Name 2:

Address:

Date of Birth:

Telephone Number:

Name 3:

Address:

Date of Birth:

Telephone Number:

Name 4:

Address:

Date of Birth:

Telephone:

Appendix 20; Control response form

Study Number

Teenage Meningococcal Disease Study Response Form

Please fill in this form and post it back to the study centre in the stamped addressed envelope provided. Alternatively you can ring the study doctor Dr Jo Tully on either of these numbers; **020- 7905 2340 or 020-72429789 Ext 2443**. You can also e-mail us j.tully@ich.ucl.ac.uk If you do not wish to take part you do not have to provide us with your contact details.

Many thanks.

I agree to take part in this study ☐

I agree only to allow you to check with my doctor if I have had the meningitis vaccine

I am unable to take part in this study & do not wish to be phoned ☐

My name is
.....

My address is
.....
.....

My home telephone number is
.....

My work telephone number is
.....

Appendix 21; Thanks to case consultant

Dear Dr

Re; Meningococcal disease in adolescence; an integrated social, biological and psychological investigation

Patient details;

.....

I am writing to thank you and the members of your team for your assistance with the study we are running on meningococcal disease in teenagers.

You kindly gave us permission to recruit one of your patients to the above study. A copy of the summary of the study protocol should have reached you.

In order to run the study we required a nose/throat swab and a serum sample from the patient in the first 48 hours following admission which you were able to provide us with. This will, we hope, provide us with data concerning recent viral infection as a predisposing factor to meningococcal infection. We have also requested samples for meningococcal serology and PCR. We will be following the patient up at 14 days post-admission to repeat the blood samples and to administer a questionnaire concerning social behaviour and psychological variables. If there are any results that your team are interested in obtaining then I would be happy to try and provide those for them.

There is a small amount of information that we require regarding laboratory test results. I have consent from the patient to access his/her medical records.

I have enclosed a response form with a stamped addressed return envelope, and would be extremely grateful if you would be able to provide us with these details. If you do not have the time to sift through the notes, a photocopy of the relevant section of the notes or laboratory reports would be equally helpful.

Thank you very much for helping us with this study and I look forward to hearing from you shortly.

Yours sincerely,

Dr Jo Tully, Clinical research Fellow

Tel; 0171 2429789 Ext 2443

e-mail; j.tully@ich.ucl.ac.uk

Please complete as fully as possible and return in the envelope provided.

- Name of patient

.....

- Name and address of admitting hospital

.....

.....

.....

- Name of consultant

.....

- Date of admission

- Admitted to ITU Yes ☐ No ☐

- Length of stay on ITU (no. of nights)

- Was this a case of (please tick box);

Meningococcal meningitis ☐

Meningococcal septicaemia ☐

Both meningitis and septicaemia ☐

- Microbiological results

	Positive	Negative	Not taken	Unknown
Blood culture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CSF culture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rapid Ag	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gram stain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Throat/nose swab	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PCR	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

- Group/ Type of meningococcus

.....

- GMSPS score if recorded (The Glasgow Meningococcal Septicaemia Prognostic Score)

Appendix 22; Thanks to GP

Dear Dr

Many thanks for assisting me in recruitment for the national study that is currently running to look at the risk factors for development of meningococcal disease in adolescence.

Your patient was admitted with meningococcal disease and you have kindly provided me with a control from your list

.....
... ..

I am writing to confirm both these subjects involvement with the research work.

No pharmacological intervention is being undertaken.

If you have any questions about your patients' involvement in this study, please do not hesitate to contact me.

Yours sincerely,

Dr. Joanna Tully

Clinical Research Fellow, Institute of Child Health

Tel; 0171-242 9789 Ext. 2443

e-mail; j.tully@ich.ucl.ac.uk

Appendix 23; Thanks to subject

Dear

I am just writing to thank you for all your help with the teenage meningococcal disease study. It is much appreciated.

We hope that the results will be published in early winter of the year 2001.

If you have any more questions you want to ask at any time, please feel free to give me a call.

May we wish you all the best for the future.

Yours sincerely,

Dr Jo Tully

Teenage meningitis study team

MENINGOCOCCAL DISEASE IN ADOLESCENCE;
AN INTEGRATED SOCIAL, BIOLOGICAL AND
PSYCHOLOGICAL INVESTIGATION

Questionnaire for young people

Institute of Child Health

1998 to 2000

INFORMATION

This is a questionnaire that is to be used by a trained clinical research fellow.

It will be completed together with the respondent.

It is designed to address risk factors that might explain why young people between the ages of 15 and 19 years of age seem to be particularly prone to developing meningococcal disease (meningitis and septicaemia).

Respondents are not required to answer any questions that they do not wish to.

All the information that is contained in this form is strictly confidential.

DEFINITIONS

We have used the following definitions for the purpose of this study.

All definitions will be explained to the interviewee.

1. **HOUSEHOLD** These are the people with whom you live and with whom you share facilities (kitchen, bathroom, living room) and meals. We have used the same definition used for decisions regarding prophylaxis by departments of public health. We have not used the General Household Survey's definition as it does not make adequate provision for the student population. If the subject has recently changed residence (within the two week period), we will consider the most recent unless that is for less than two nights.
2. **CLOSE FRIENDS AND REGULAR CLOSE CONTACTS** These are as defined by the subject. A decision was made not to define these rigorously.
3. **HALLS OF RESIDENCE** This is a communal form of living that is associated with a common educational institution.
4. **HOSTEL** This is a communal form of living not associated with any common educational institution. The residents are likely to have less intimate contact with one another.
5. **SOCIALISING** People with whom you spend time with solely for pleasurable purposes.
6. **UNEMPLOYED** Those who are out of work and have looked for work in the four weeks before interview, or would have but for temporary sickness or injury, and are available to start work in the two weeks following the interview.
7. **PRECEDING ILLNESS** This is an illness that resolved completely or partially prior to meningococcal disease developing.

SECTION A INTRODUCTION

In this section we will look at some basic personal details.

- A1. **Study number of interviewee**
- A2. **Set number of interviewee**
- A3. **Date of interview**
- A4. **Interview with**
- | | | | |
|-----------|--------------------------|-----------|--------------------------|
| Case | <input type="checkbox"/> | Control 2 | <input type="checkbox"/> |
| Control 1 | <input type="checkbox"/> | Control 3 | <input type="checkbox"/> |
- A5. **Age**
- A6. **Date of birth**
- A7. **Sex**
- | | | | |
|------|--------------------------|--------|--------------------------|
| Male | <input type="checkbox"/> | Female | <input type="checkbox"/> |
|------|--------------------------|--------|--------------------------|
- A8. **How would you best describe your ethnic origin?**
- | | | | |
|--------------------|--------------------------|-------------|--------------------------|
| White | <input type="checkbox"/> | Pakistani | <input type="checkbox"/> |
| Black African | <input type="checkbox"/> | Bangladeshi | <input type="checkbox"/> |
| Black Caribbean | <input type="checkbox"/> | Chinese | <input type="checkbox"/> |
| Black other | <input type="checkbox"/> | Indian | <input type="checkbox"/> |
| Other ethnic group | <input type="checkbox"/> | | |
- If other ethnic group or black other please specify
-
- A9. **Main address where usually resident**
-
-
- Postcode Tel no.....
- A10. **Address when first became unwell (cases)
or in last 2 weeks (controls)**
-
-
- Postcode

SECTION B YOUR HEALTH

In this section I am going to ask questions about your illness (cases) and your general health (cases and controls). If you are a case I will ask you about the 2 weeks before you became unwell with meningococcal disease and if you are a control I will ask you about the last 2 weeks.

If you are a control, go to Question B3

B1. When did you first become unwell with meningococcal disease?

Date

B2. What were the first symptoms?

Fever	<input type="checkbox"/>	Loss of consciousness	<input type="checkbox"/>
Shivery, hot/cold	<input type="checkbox"/>	Unusual tiredness	<input type="checkbox"/>
Headache	<input type="checkbox"/>	Loss of appetite	<input type="checkbox"/>
Skin rash	<input type="checkbox"/>	Nausea	<input type="checkbox"/>
Neck stiffness	<input type="checkbox"/>	Vomiting	<input type="checkbox"/>
Muscle/joint aches	<input type="checkbox"/>	Sore throat	<input type="checkbox"/>
Back pain	<input type="checkbox"/>	Cough	<input type="checkbox"/>
Swollen joints	<input type="checkbox"/>	Runny nose	<input type="checkbox"/>
Photophobia	<input type="checkbox"/>	Abdominal pain	<input type="checkbox"/>
Blurred vision	<input type="checkbox"/>	Diarrhoea	<input type="checkbox"/>
Dizziness or fainting	<input type="checkbox"/>		

B3. Ask the case;

In the two weeks before you had meningococcal disease, did you have any other illnesses that started in these two weeks?

Yes ☐ No ☐

Ask the control;

Have you been unwell in any way in the last 2 weeks?

Yes ☐ No ☐

Were you unwell (2) in any way in the week beginning.....?

Yes ☐ No ☐

If NO go to Question B8

B4. What was the date/s that this illness started? 1
2

B5. Did you have any of the following symptoms?

	1	2		1	2
Fever	<input type="checkbox"/>	<input type="checkbox"/>	Loss of consciousness	<input type="checkbox"/>	<input type="checkbox"/>
Shivery, hot/cold	<input type="checkbox"/>	<input type="checkbox"/>	Unusual tiredness	<input type="checkbox"/>	<input type="checkbox"/>
Headache	<input type="checkbox"/>	<input type="checkbox"/>	Loss of appetite	<input type="checkbox"/>	<input type="checkbox"/>
Rash	<input type="checkbox"/>	<input type="checkbox"/>	Nausea	<input type="checkbox"/>	<input type="checkbox"/>
Neck stiffness	<input type="checkbox"/>	<input type="checkbox"/>	Vomiting	<input type="checkbox"/>	<input type="checkbox"/>
Muscle/joint aches	<input type="checkbox"/>	<input type="checkbox"/>	Sore throat	<input type="checkbox"/>	<input type="checkbox"/>
Back pain	<input type="checkbox"/>	<input type="checkbox"/>	Cough	<input type="checkbox"/>	<input type="checkbox"/>
Swollen joints	<input type="checkbox"/>	<input type="checkbox"/>	Runny nose	<input type="checkbox"/>	<input type="checkbox"/>
Photophobia	<input type="checkbox"/>	<input type="checkbox"/>	Abdominal pain	<input type="checkbox"/>	<input type="checkbox"/>
Blurred vision	<input type="checkbox"/>	<input type="checkbox"/>	Diarrhoea	<input type="checkbox"/>	<input type="checkbox"/>
Dizziness or fainting	<input type="checkbox"/>	<input type="checkbox"/>			

B6. Did you see a doctor because of either of these illnesses?

Yes 1 ☐ 2 ☐ No 1 ☐ 2 ☐

If YES, what was the diagnosis? 1.....

2.....

B7. Did you receive any treatment for either of these illnesses?

Yes 1 ☐ 2 ☐ No 1 ☐ 2 ☐

If YES, please specify

The next set of questions relate to your general health.

B8. Do you see a doctor on a regular basis for any illness?

(Excluding meningococcal disease)

Yes ☐ No ☐

If YES, prompt for details

.....

B9. Have you taken any antibiotics in the last two weeks/ the 2 weeks before becoming ill?

Yes ☐ No ☐ Not sure ☐

SECTION C YOUR OCCUPATION

This section of the interview looks at your current occupation.
We will ask about your main occupation, but will also want to know about any part-time, evening or weekend work.

C1. What is your current main occupation?

- | | | | |
|----------------|--------------------------|-----------------|--------------------------|
| School student | <input type="checkbox"/> | College student | <input type="checkbox"/> |
| Home duties | <input type="checkbox"/> | Unemployed | <input type="checkbox"/> |
| Self-employed | <input type="checkbox"/> | Employed | <input type="checkbox"/> |
| Other | <input type="checkbox"/> | | |

If OTHER, please specify

C2. If employed or self-employed, what is the job that you do?

(Prompt for type of work done Eg. Cleaner, clerk and type of course or study)

.....

C3. Do you have any other job?

- Yes ☐ No ☐

If YES, please give details

C4. Please describe your place or places of work (tick one or more)

- | | | | |
|------------|--------------------------|------------------------|--------------------------|
| Home | <input type="checkbox"/> | Office | <input type="checkbox"/> |
| Workshop | <input type="checkbox"/> | Factory | <input type="checkbox"/> |
| School | <input type="checkbox"/> | College/
University | <input type="checkbox"/> |
| Shop | <input type="checkbox"/> | Outdoors | <input type="checkbox"/> |
| Pub or bar | <input type="checkbox"/> | Other | <input type="checkbox"/> |

If other please describe

If unemployed,

C5. What was your occupation before becoming unemployed?

- | | | | |
|----------------|--------------------------|-----------------|--------------------------|
| School student | <input type="checkbox"/> | College student | <input type="checkbox"/> |
| Employed | <input type="checkbox"/> | Self-employed | <input type="checkbox"/> |
| Other | <input type="checkbox"/> | | |

If other please specify

C6. If employed or self-employed, what was your job?

.....

SECTION D YOUR ACCOMMODATION

I am now going to ask you about the place where you have lived in the last two weeks. This does not include overnight stays or weekend stays but must be a place that you live Eg. Home with parents, rented home or college. If there are two places that you have lived, we will ask about the most recent one unless you have been there for less than two nights.

D1. What type of accommodation have you been living in?

- | | | | |
|--------------------|--------------------------|---------------------|--------------------------|
| House | <input type="checkbox"/> | Flat | <input type="checkbox"/> |
| Maisonnette | <input type="checkbox"/> | Caravan/Mobile home | <input type="checkbox"/> |
| Hostel | <input type="checkbox"/> | Bed & Breakfast | <input type="checkbox"/> |
| Halls of residence | <input type="checkbox"/> | Other | <input type="checkbox"/> |

If OTHER, please specify

D2. How many people live in this household?

D3. How many rooms are there in this household?

(Include the kitchen but exclude the bathroom, loo and hall)

The following questions apply to students

D4. . If you are a student which college term are you currently in?

The following questions apply to students who are living in halls of residence. If you are not, please go to question E1.

D5. How many students live in your hall of residence?

(This is the total number in all blocks)

D6. Are your halls of residence mainly self-catering halls or are meals provided for you (catered)?

- Self-catering ☐
- Meals provided ☐

D7. Do you have self-catering facilities?

Yes ☐ No ☐

If YES, how many people do you share them with?

D8. How many times in the last two weeks have you eaten in a communal area?

More than 7 times ☐

1-7 times ☐

Never ☐

SECTION E YOUR HOUSEHOLD

I am now going to ask you some questions about the people that you live with and have close contact with. Many of the questions will relate to the same 2 week period as before.

E1. Please give details of all the people that live with you

(For household members include all the people who normally live with you and share meals with you. If you are a student in halls of residence, include only those with whom you share facilities Eg. Kitchen, bathroom)

INITIAL	AGE	OCCUPATION	RELATIONSHIP TO SUBJECT	COMMENTS (Smokers?)

E2. Please give details of any guests that have stayed with you in the last two weeks

(Include only those who have stayed overnight in your household or if you are in Halls of Residence, your bedroom)

INITIALS	AGE	REL. TO SUBJECT	COMMENTS (Smokers?)

E3. Please give details of all the people in the last two weeks with whom you have had close contact other than your household members and guests

(At least once a week, sharing a family meal/bedroom etc. not including work or school contacts Eg. grandparents, other close relatives, partner.)

[illegible]

E4. In the last 2 weeks, have you spent any nights away from the usual place where you live?

(Eg. Social, business, holiday)

Yes ☐ No ☐

E5. In the last 2 weeks, how many people have you kissed?

No. snogging contacts ☐ ☐

Dates _____

No. lip kissing contacts ☐ ☐

E6. In the last 2 weeks have you shared a bed with anyone?

(This refers to sleepovers at friend's houses, staying over etc. as well as partners)

Yes ☐ No ☐

If YES, how many people is this with

E7. In the last two weeks have you shared a bedroom?

Yes ☐ No ☐

If YES, how many times?

SECTION F SMOKING, DRINKING AND DRUGS

In this next section I am going to ask you questions about you and your households smoking habits.

I will also ask you some questions about how much you drink and whether you have used recreational drugs.

Remember, all information contained in this form is strictly confidential.

TOBACCO SMOKING

F1. Are you currently a smoker?

Yes ☐ No ☐

If YES, how many times do you smoke a day?

Less than 10 ☐

11 to 19 ☐

20 to 29 ☐

30 or more ☐

If YES, go to Question F4

F2. Are you an ex-smoker?

Yes ☐ No ☐

If YES, when did you give up?

F3. In the 2 weeks before you were admitted to hospital (cases) or the last two weeks (controls), have you smoked any cigarettes or cigars at all?

Yes ☐ No ☐

If YES, how many?

F4. In the 3 days before you were admitted to hospital (cases) or in the last 3 days (controls), how many cigarettes have you smoked?

F5. How many people in your household smoke other than yourself?

If NONE, go to question F8

F6. Do any of them smoke inside the house?

Yes ☐ No ☐

F7. Of those who smoke, how many cigarettes a day does each person smoke altogether and how many cigarettes a day does each person smoke inside the house?

HOUSEHOLD MEMBER	NO. SMOKED PER DAY	NO. SMOKED INSIDE HOUSE

Please go to question F9

F8. Have you been exposed to smoke in your household in the last 2 weeks?

Yes ☐ No ☐

If YES, how many times has this occurred?

(Prompt for dinner parties, visitors etc)

F9. Are you exposed to smoke at your place of work?

Yes ☐ No ☐ Not applicable ☐

F10. How many of your close contacts are regular smokers incl. household members?

ALCOHOL

F11. Have you drunk any alcohol in the last 2 weeks?

Yes ☐ No ☐

If NO, go to question F14

F12. How much alcohol do you think you have drunk in the last 2 weeks?

Beer/lager/cider (no.of half pints or bottles)

Wine (no. of glasses)

Spirits Eg. Gin, vodka (no. single measures or "shots")

Others Eg.Alcopops, port, sherry etc

F13. How many times have you had a hangover in the last two weeks?

F14. How many times have you been on a drinking binge/ had too much to drink, but not had a hangover in the last two weeks?

DRUGS

F15. Do you use any recreational drugs on a regular basis? (Once a week or more)

(Prompt for examples)

Yes ☐ No ☐

F16. How many times have you used recreational drugs in the last 2 weeks?

If you have not used recreational drugs in the last two weeks, go to QG1

F17 .In the last two weeks which of the following have you used and were any of them sniffed or smoked?

DRUG		SNIFFED OR SMOKED? (Tick if yes)
Glue, Tippex, Other solvents	<input type="checkbox"/>	<input type="checkbox"/>
Marijuana	<input type="checkbox"/>	<input type="checkbox"/>
Cocaine/Crack cocaine	<input type="checkbox"/>	<input type="checkbox"/>
Heroin	<input type="checkbox"/>	<input type="checkbox"/>
Others	<input type="checkbox"/>	<input type="checkbox"/>

Of others, please give details

SECTION G LEISURE ACTIVITIES

We now want to look at the ways in which you spend your spare time.

We would like to know where you have been in the last two weeks and whether these places are smoky places.

G1. In the past 2 weeks, how many times have you visited any of the following places?

	Not Visited	1-7 times	>7 times	Every day	Smoky? (Tick if smoky)
Pub or bar	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Nightclub, disco house party or rave	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Youthclub	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Group activities (Guides, nightclass)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Church/church Groups	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Friends room or house	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Leisure or sports centre, swimming pool	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Student common room	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SECTION H SPORTING ACTIVITIES

This section is asking about the sports and other physical activities that you have taken part in over the last two weeks.

These sports and activities should include organised sports such as team sports, tennis and swimming as well as activities that you do not formally organise such as cycling to work, jogging, playing football during breaks at school, playing snooker in the pub etc.

We also want you to think about how much effort you think that particular activity required. If you think it didn't take much effort and you didn't feel out of breath or sweaty, then it was a light activity. If you felt a little out of breath then it was a moderate activity. If you felt out of breath and tired then it was vigorous and if you were very sweaty, out of breath and tired then it was very vigorous.

Please fill in the following table, thinking about the two weeks before you got ill if you are a case or the last two weeks if you are a control.

H1.

[illegible]

H2. Is this two week period different from your usual level of exercise or activity?

Yes ☐ No ☐

If YES, in what way is this two week period different?

It is more than the usual amount of exercise that I do ☐

It is less than the usual amount of exercise that I do ☐

SECTION J THE STRESSES IN YOUR LIFE

We are interested in finding out the kinds of things that cause you stress.

In the following section you will find statements to which you can either answer Yes or No. We want you to think back over the last twelve months and decide whether any of these things have happened to you or your family. For the purpose of this questionnaire your family includes your brothers, sisters, parents and grandparents and also any step-siblings or half-siblings that you live with.

If you are unsure who to include, check with me.

If you decide that this event has happened to you or your family in the last year, then tick YES. If it has not happened or happened more than a year ago, tick NO.

We would also like to know how much this event upset or distressed you at the time that it happened. Underneath the item is a scale that ranges from not distressing to extremely distressing. Decide the position on the line that best describes your feelings at the time and put a cross there. Do this for all the items.

Remember, this information is strictly confidential. Please be as truthful as you can.

A. FAMILY CHANGES

J1. A member of the family started a new business Yes ☐

No ☐

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J2. A parent resigned from work or lost their job Yes ☐

No ☐

If YES, how distressing did you find this?

Not distressingExtremely distressing

J3. Parents separated or divorced

Yes ☐No ☐

If YES, how distressing did you find this?



J4. Parents or one parent remarried

Yes ☐No ☐

If YES, how distressing did you find this?

Not distressing Extremely distressing

J5. A family member was found to have a learning disorder

Yes ☐No ☐

If YES, how distressing did you find this?

Not distressing Extremely distressing

J6. Family member got married

Yes ☐No ☐

If YES, how distressing did you find this?

Not distressing Extremely distressing

J7. Parents adopted a child

Yes ☐No ☐

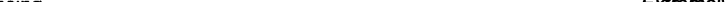
If YES, how distressing did you find this?

[illegible]

J8. A family member started at secondary school

Yes ☐No ☐

If YES, how distressing did you find this?



J9. A family member transferred to a new school

Yes ☐

No ☐

If YES, how distressing did you find this?

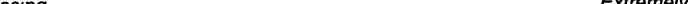
Not distressing Extremely distressing

J10. A parent started at college

Yes ☐

No ☐

If YES, how distressing did you find this?



J11. **Brother or sister moved away from home**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

J12. **Brother or sister started college, training or armed forces**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

J13. **Parent(s) started or changed to a new job**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

J14. **Family moved to a new home**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

B. FAMILY SEXUAL MATTERS

J15. A member of your family had an unplanned pregnancy

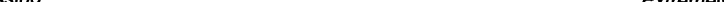
Yes

□

No

1

If YES, how distressing did you find this?



J16. A member of your family had an abortion

Yes

7

No

1

If YES, how distressing did you find this?

Not distressing Extremely distressing

J17. A brother or sister was born

Yes

1

No

☐

If YES, how distressing did you find this?

Not distressing Extremely distressing

J18. A teenage member of your family started an intimate relationship

Yes

9

No

□

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

C. FAMILY LOSSES

J19. The family started claiming social security benefits

Yes

☐

No

If YES, how distressing did you find this?

Not distressing Extremely distressing

J20. Family property was lost or damaged due to burglary, fire etc.

Yes

1

No

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J21. Brother or sister died

Yes

☐

No

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J22. Parent died

Yes

7

No

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J23. **A close relative died**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

J24. **Close friend or another family member died**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

J25. **Family member or close friend attempted or committed suicide**

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→
Not distressing *Extremely distressing*

D. FAMILY RESPONSIBILITIES AND STRAINS

J26. A family member became seriously ill or was injured, but not hospitalised

Yes ☐No ☐

If YES, how distressing did you find this?

←-----→

Not distressing *Extremely distressing*

J27. Family member was hospitalised

Yes ☐No ☐

If YES, How distressing did you find this?

Not distressingExtremely distressing

J28. Family member became disabled or developed long-term illness

Yes ☐No ☐

If YES, how distressing did you find this?

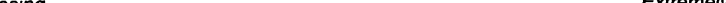
[illegible]

J29. Family member has emotional problems

Yes ☐

No ☐

If YES, how distressing did you find this?



J30. Grandparent(s) became seriously ill

Yes ☐

7

No

9

If YES, how distressing did you find this?

[illegible]

J31. Parent(s) have more responsibility to take care of grandparent(s)

Yes ☐

☐

No

7

If YES, how distressing did you find this?

Not distressing Extremely distressing

J32. Family member ran away

Yes ☐

☐

No

7

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J33. Family ran into more debt

Yes ☐

1

No

7

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J34. A recent increase in the family's living expenses

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→

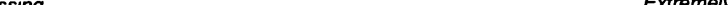
Not distressing *Extremely distressing*

J35. A parent or parents have spent more time away from the family

Yes ☐

No ☐

If YES, how distressing did you find this?

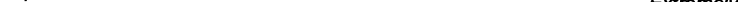


J36. Child or teenage member resists doing things with the family

Yes ☐

No ☐

If YES, how distressing did you find this?



J37. Increase in arguments between the parents

Yes ☐

No ☐

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J38. Children or teenagers arguing more with each other

Yes

1

No

4

If YES, how distressing did you find this?

Not distressing Extremely distressing

**J39. Parent(s) and teenager(s) have more arguments over using car/
staying out late**

Yes

□

No

☐

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J40. Parent(s) and teenager(s) have more arguments over choice of friends/ social activities

Yes

☐

No

☐

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J41. Parent(s) and teenager(s) have more arguments over attending religious events

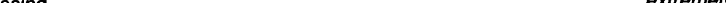
Yes

1

No

7

If YES, how distressing did you find this?



J42. Parent(s) and teenager(s) have more arguments over appearance (Eg. Hairstyle, earrings, tattoos etc)

Yes

5

No

4

If YES, how distressing did you find this?

←————→

Not distressing *Extremely distressing*

J43. More arguments about getting the jobs done at home

Yes

No

If YES, how distressing did you find this?

Not distressing Extremely distressing

**J44. Pressure on school members of the family to do well at school/
in sports**

Yes

7

No

7

If YES, how distressing did you find this?

[illegible]

E. STRESSES AT SCHOOL AND SUBSTANCE USE

J45. A family member is using drugs (not given by the doctor)

Yes

7

No

7

If YES, how distressing did you find this?

[illegible]

J46. A family member drinks too much alcohol

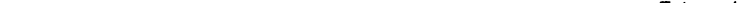
Yes

7

No

7

If YES, how distressing did you find this?



Yes ☐

No ☐

Not distressing Extremely distressing

Yes ☐

No ☐

Not distressing Extremely distressing

F. FAMILY LEGAL STRESSES

Yes ☐

No ☐



Yes ☐

No ☐

[illegible]

SECTION K YOUR SOCIAL SUPPORT

The following questions ask about people in your environment who provide you with help or support. Each question has two parts. For the first part, list all the people you know, excluding yourself, whom you can count on for help or support in the situations described. Give the person's initials and their relationship to you. Do not list more than nine people for each question.

For the second part, circle how satisfied you are with the level of support you get from all the people as a whole.

If you feel there is no support available to you for a question, then tick "no-one" but still answer to tell us how satisfied you are with that.

Please answer all questions as best you can. All your responses will be kept confidential.

K1. Whom can you really count on to be dependable when you need help?

No-one ☐ 1. 4. 7.
2. 5. 8.
3. 6. 9.

How satisfied?

6-very satisfied 5-fairly satisfied 4-a little satisfied
3-a little dissatisfied 2-fairly dissatisfied 1-very dissatisfied

K2. Whom can you really count on to help you feel more relaxed when you are under pressure or tense?

No-one ☐ 1. 4. 7.
2. 5. 8.
3. 6. 9.

How satisfied?

6-very satisfied 5-fairly satisfied 4-a little satisfied
3-a little dissatisfied 2-fairly dissatisfied 1-very dissatisfied

K3. Who accepts you totally, including both your best and worst points?

No-one	1.	4.	7.
	2.	5.	8.
	3.	6.	9.

How satisfied?

6-very satisfied	5-fairly satisfied	4-a little satisfied
3-a little dissatisfied	2-fairly dissatisfied	1-very dissatisfied

K4. Whom can you really count on to care about you, regardless of what is happening to you?

No-one	1.	4.	7.
	2.	5.	8.
	3.	6.	9.

How satisfied?

6-very satisfied	5-fairly satisfied	4-a little satisfied
3-a little dissatisfied	2-fairly dissatisfied	1-very dissatisfied

K5. Whom can you really count on to help you feel better when you are feeling generally down-in-the-dumps?

No-one	1.	4.	7.
	2.	5.	8.
	3.	6.	9.

How satisfied?

6-very satisfied	5-fairly satisfied	4-a little satisfied
3-a little dissatisfied	2-fairly dissatisfied	1-very dissatisfied

K6. Whom can you count on to console you when you are very upset?

No-one	1.	4.	7.
	2.	5.	8.
	3.	6.	9.

How satisfied?

6-very satisfied	5-fairly satisfied	4-a little satisfied
3-a little dissatisfied	2-fairly dissatisfied	1-very dissatisfied

Do you have any objections to us contacting you in the future as part of a follow-up study?

I have no objection to being contacted ☐

I do not wish to be contacted ☐

SECTION L HOSPITAL DETAILS

For the interviewer to complete from hospital records if possible.

L1. Name and address of admitting hospital
.....
.....
.....

L2. Name of consultant

L3. Date of admission

L4. Admitted to ITU Yes ☐ No ☐

L5. Length of stay on ITU (no. nights)

L6. Was this a case of;

Meningococcal meningitis ☐

Meningococcal septicaemia ☐

Both meningitis and septicaemia ☐

L7. Microbiological results

	Positive	Negative	Not taken	Unknown
Blood culture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
CSF culture	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Rapid Ag	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gram stain	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Throat/nose swab	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
PCR	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

L8. Group/ Type of meningococcus

L9. GMSPS score if recorded

L10. Signature of interviewer

MENINGOCOCCAL DISEASE IN ADOLESCENCE;
AN INTEGRATED SOCIAL, BIOLOGICAL AND
PSYCHOLOGICAL INVESTIGATION

Questionnaire for the head of household

Institute of Child Health
1998 to 2000

INFORMATION

This questionnaire is for use by a designated clinical research fellow.

It is designed for the parents of a young person who has been affected by meningococcal disease.

It is an investigation into possible risk factors associated with the development of meningococcal disease within the adolescent population.

All information on this form is strictly confidential.

SECTION A PERSONAL DETAILS

AA1. Date of interview

--	--	--	--	--	--

AA2. Relationship to case/control

AA3. Case/control number

--	--	--	--

AA4. Other relevant persons interviewed

Relationship to case/control

AA5. Address
.....
.....

Postcode

AA7. Case/control's GP's address
.....
.....

SECTION B YOUR FAMILY'S HEALTH

This section deals with your household's health.

BB1. Is case/control seen regularly by a doctor for any illness?

Yes ☐ No ☐

Please describe

BB2. Is there a family history of meningitis?

(Parents, brothers, sisters, grandparents, uncles, aunts, nephews, nieces)

Yes ☐ No ☐ Not sure ☐

If YES,

RELATIONSHIP TO SUBJECT	TYPE OF MENINGITIS	DATE

BB3. Has the case/control had meningitis before?

Yes ☐ No ☐

If YES, what type of meningitis was it?

Meningococcal ☐
 Other bacterial ☐ Specify
 Viral ☐
 Other ☐ Specify
 Not sure ☐

BB4. Has the case/control had meningococcal disease before?

Yes ☐ No ☐

BB5. Has the case/control ever been vaccinated against meningitis?

Yes ☐ No ☐ Not sure ☐

BB6. Has the case/control ever been vaccinated against flu?

Yes ☐ No ☐ Not sure ☐

BB7. How much did case/control weigh at birth?
(In kg to two decimal places)

BB8. How many weeks of pregnancy had been completed when case/control was born?

BB9. Was the case/control breast fed as a baby?

Yes ☐ No ☐ Not sure ☐

If Yes, for how long?	Less than 1 month	<input type="checkbox"/>
	1-6 months	<input type="checkbox"/>
	More than 6 months	<input type="checkbox"/>
	Not sure	<input type="checkbox"/>

SECTION C YOUR OCCUPATION AND HOME

This section of the questionnaire is about the head of the households occupation where the case or control is not the head of the household.

CC1. What is the current occupation of the head of the household?

Student	<input type="checkbox"/>	Houseduties	<input type="checkbox"/>
Employed	<input type="checkbox"/>	Self-employed	<input type="checkbox"/>
Unemployed	<input type="checkbox"/>	Retired	<input type="checkbox"/>
Other	<input type="checkbox"/>	Please specify	

CC2. If unemployed, please state for how long

.....

CC3. In your current job please describe your work

(If retired or unemployed, please state last occupation)

.....

CC4. Is there a car or van normally available for use by you?

Yes ☐ No ☐

If YES, how many? ☐

CC5. For the house that you live in, what type of ownership do you have?

Owner occupied	<input type="checkbox"/>	Council owned	<input type="checkbox"/>
Privately rented	<input type="checkbox"/>	Other	<input type="checkbox"/>

UNIVERSITY OF LONDON

SENATE HOUSE. MALET STREET, LONDON, WC1E 7HU



REPRODUCTION OF THESES

A thesis which is accepted by the University for the award of a Research Degree is placed in the Library of the College/Institution and in the University of London Library. The copyright of the thesis is retained by the author.

As you are about to submit a thesis for a Research Degree, you are required to sign the declaration below. This declaration is *separate* from any which may be made under arrangements with the College at which you have *pursued* your course (for internal candidates only). The declaration will be destroyed if your thesis is not approved by the examiners, being either rejected or referred for revision.

Academic Registrar

To be completed by the candidate

NAME IN FULL (Block Capitals)

JOANNA MARY TULLY

TITLE OF THESIS

RISK AND PROTECTIVE FACTORS FOR THE DEVELOPMENT OF
MENINGOCOCCAL DISEASE IN ADOLESCENCE : A BIOPSYCHOSOCIAL
INVESTIGATION

DEGREE FOR WHICH THESIS IS PRESENTED

MD

DATE OF AWARD OF DEGREE (To be completed by the University)

31 DEC 2006

DECLARATION

1. I authorise that the thesis presented by me in *[2005] for examination for the MPhil/PhD Degree of the University of London shall, if a degree is awarded, be deposited in the library of the appropriate College and in the University of London Library and that, subject to the conditions set out below, my thesis be made available for public reference, inter-library loan and copying.
2. I authorise the College or University authorities as appropriate to supply a copy of the abstract of my thesis for inclusion in any published list of theses offered for higher degrees in British universities or in any supplement thereto, or for consultation in any central file of abstracts of such theses.
3. I authorise the College and the University of London Libraries, or their designated agents, to make a microform or digital copy of my thesis for the purposes of inter-library loan and the supply of copies.
4. I understand that before my thesis is made available for public reference, inter-library loan and copying, the following statement will have been included at the beginning of my thesis: The copyright of this thesis rests with the author and no quotation from it or information derived from it may be published without the prior written consent of the author.
5. I authorise the College and/or the University of London to make a microform or digital copy of my thesis in due course as the archival copy for permanent retention in substitution for the original copy.
6. I warrant that this authorisation does not, to the best of my belief, infringe the rights of any third party.
7. I understand that in the event of my thesis being not approved by the examiners, this declaration would become void.

*Please state year.

DATE 4TH NOVEMBER 2004 SIGNATURE

Note: The University's Ordinances make provision for restriction of access to an MPhil/PhD thesis and/or the abstract but only in certain specified circumstances and for a maximum period of two years. If you wish to apply for such restriction, please enquire at your College/Institution about the conditions and procedures. External Students should enquire at the Research Degree Examinations Office, Room 261, Senate House.

**THIS DECLARATION MUST BE COMPLETED AND RETURNED WITH THE
EXAMINATION ENTRY FORM**